CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

W. F. GEDDES, Editor-in-Chief
R. J. TARLETON, Managing Editor
EUNICE R. BROWN, Assistant Editor
} 1955 University Avenue, St. Paul 4, Minnesota

Editorial Board: Welker G. Bechtel, B. Marlo Dirks, G. N. Irvine, Robert A. Larsen, Dale K. Mecham, Byron S. Miller, C. E. Rist, and Thomas J. Schoch.

CONTENTS

PAGE

The Subsieve-Size Fractions of a Hard Red Spring Wheat Flour Produced by Air Classification. *Rezsoe Gracza* 579

Milling Properties of Wheat in Relation to Pearling, Scouring, and Impaction. *G. M. Grosh, J. A. Shellenberger, and E. P. Farrell* 593

The "Remix" Baking Test. *G. N. Irvine and Marion E. McMullan* 603

Some Chemical Changes Which Accompany the Browning of Canned Bread during Storage. *Russell J. Stenberg and W. F. Geddes* 614

Studies on Accelerated Browning in Starch Pastes Containing Various Bread Ingredients. *Russell J. Stenberg and W. F. Geddes* 623

Identification of Carbonyl Compounds in an Ethanol Extract of Fresh White Bread. *H. Ng, D. J. Reed, and J. W. Pence* 638

Particle Size in Aqueous Starch Dispersions. *G. E. Babcock, R. Tobin, R. J. Dimler, and F. R. Senti* 645

Preparation and Properties of Hydroxyethylated High-Amylose Corn Starch. *J. C. Rankin, J. G. Rall, C. R. Russell, and F. R. Senti* 656

A Note on the Determination of Gelatinization Temperatures of Rice Varieties. *J. V. Halick, H. M. Beachell, J. W. Stansel, and H. H. Kramer* 670

Sedimentation Value as an Index of Dough-Mixing Characteristics in Early-Generation Wheat Selections. *Lawrence Zeleny, W. T. Greenaway, Georgia M. Gurney, C. C. Fifield, and Kenneth Lebsock* 673

Communication to the Editor: Presence and Probable Role of Thioctic Acid in Wheat Flour. *Leland Dahle and Betty Sullivan* 679

Editorial Policy and Suggestions to Authors 682

Second-class postage paid at Minneapolis, Minn. Published at 500 S. 5th St., Minneapolis, Minn. Subscription rates $15.00 per year. Foreign postage, $1.00 extra. Single copies, $2.75; foreign, $2.95. Back issues, $3.25. Claims for missing numbers will not be allowed if received more than 60 days from date of mailing plus time normally required for postal delivery of journal and claim. No claims allowed when subscriber has failed to notify the Circulation Department of a change of address, or because copy is missing from subscriber's files.

Copyright © 1960 by the American Association of Cereal Chemists, Inc.

Vol. 37 September, 1960 No. 5

The BAILEY #505-SS FERMENTATION CABINET

This stainless cabinet is the successor to the Despatch Model #505 which has been on the market for some years. Many units of the re-designed model have been put into service in leading laboratories with very good success.

Walton Humidifier with constant level control

Stainless Exterior

Stainless Interior

Stainless or brass hardware

Built-in Sliding Shelf

110-120V 60C A.C. Current. Maximum load 1600 watts

¼ ton Refrigeration Unit
Extra – $380.00

Outside Cabinet Dimensions
60″ x 60″ x 24″ deep

Welded steel stand is 22″ high or will build to suit. Grey shrivel finish – stainless trim
Extra – $108.00

Model #505-SS Bailey Fermentation Cabinet – $1658.00
Est. Shipping Weight 1000 lbs.
Prices F.O.B. Lincoln, Nebr.

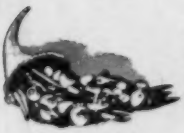
CEREAL CHEMISTS' EQUIPMENT MANUFACTURER

NATIONAL MFG. COMPANY
LINCOLN, NEBRASKA

BETA CAROTENE

IN THE SPOTLIGHT

Colors call to your appetite. Some foods are naturally attractive in color. Some must be made so. For example, butter and margarine must be a good yellow for widest appeal.



Making foods look better, making them taste better, making them nutritionally more valuable are practices everyone accepts and expects. Food processors now add pure, safe beta Carotene Roche to give natural yellow color, plus vitamin A value.

Beta carotene is a *safe* color. Beta carotene is one of nature's own coloring agents. It has been consumed in foods for thousands of years. Beta carotene is officially accepted as a food coloring in the U.S.A., Great Britain, Switzerland, and many other countries. Health, too, is served by beta carotene. Being a provitamin, it contributes vitamin A value. *Beta Carotene is the color that nourishes.*

Look for the words: "Colored With Beta Carotene" on the labels of foods you buy, such as margarine, shortening, lard, butter, cheese, salad and cooking oils, confections, yellow baked goods, beverages, and ice cream products. You can be sure that the food processors who use beta carotene have your good health in mind.

More information about beta carotene, in an interesting brochure, is yours for the asking. Just write to the Department of Education at the address below.

The Roche Technical Department is ready to help you with any problems you may have if you make pharmaceuticals or process foods. There is no obligation, of course.

FINE CHEMICALS DIVISION • **HOFFMANN-LA ROCHE INC.** • NUTLEY 10, NEW JERSEY

Roche Research and Roche Products Preserve and Protect the World's Health

ROCHE ROUND THE WORLD

AFFILIATED COMPANIES: BASEL • BOGOTA • BOMBAY • BRUSSELS • BUENOS AIRES • GRENZACH • HAVANA ISTANBUL • JOHANNESBURG • LONDON • MADRID • MEXICO CITY • MILAN • MONTEVIDEO • MONTREAL PARIS • RIO DE JANEIRO • STOCKHOLM • SYDNEY • TOKYO • AGENCIES IN OTHER COUNTRIES

ROCHE®

©1960 H-LR, INC.

MERCK PRODUCTS FOR THE FOOD INDUSTRY

Ascorbic Acid

Mercate "5"
(Isoascorbic Acid, Merck)

Citric Acid

Mercate "20"
(Sodium Isoascorbate, Merck)

Niacin

Riboflavin

Sodium Ascorbate

Sodium Citrate

Sorbitol

Thiamine

Vitamin Mixtures
for Flour, Corn Products, Farina, and Macaroni

Vitamin Wafers
for Bakery Products and Macaroni

Vitamin A Acetate

Vitamin A Palmitate

Vitamin B_6

Vitamin B_{12}

MERCK CHEMICAL DIVISION

MERCK & CO., INC. • RAHWAY, NEW JERSEY

iff FLAVORS EMPTY MORE SHELVES

Why? Because quality flavors make quantity sales.

Experienced flavor chemists at IFF can create new flavors for packaged mixes, exclusively yours . . . improve flavor stability and shelf-life . . . reduce flavor bake-out. Or if yours is an established product, IFF flavors can add new zest with renewed sales impact.

Uniform manufacturing and quality control in all of its plants throughout the world assure customers that IFF flavors, wherever ordered, remain the same from batch to batch.

For the flavor that's sales-perfect for your product, contact IFF.

iff

FLAVOR DIVISION

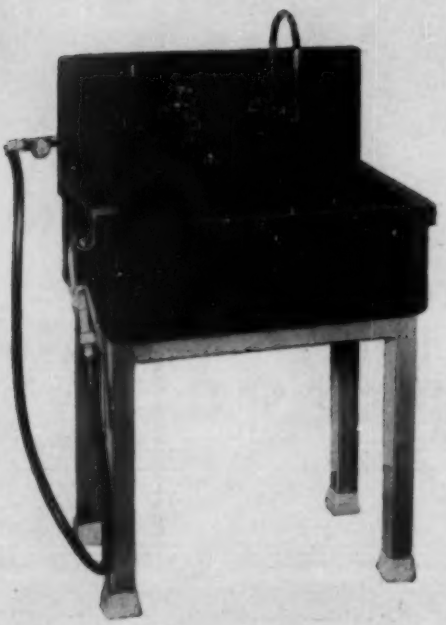
INTERNATIONAL FLAVORS & FRAGRANCES INC.

417 Rosehill Place, Elizabeth 2, N. J.

Leading Creators and Manufacturers in the World of Flavor

ARGENTINA AUSTRIA BELGIUM BRAZIL CANADA ENGLAND FRANCE GERMANY HOLLAND INDONESIA ITALY NORWAY SOUTH AFRICA SWEDEN SWITZERLAND U.S.A.

Labconco KJELDAHL SINK



B-452-C Sink, stand, chemical lead waste plug and tailpiece, single laboratory faucet with swinging gooseneck spout, flask washer, six foot hose and valve as shown above. Price **$235.10**

This sink is ideal for any laboratory concerned with Kjeldahl determinations. The sink itself is one piece chemical porcelain, offers maximum strength, thermal shock resistance and chemical inertness. The smooth interior with rounded corners is easy to keep clean and the new blue-grey glaze is attractive in any laboratory. The all steel support stand has leveling adjustments concealed by aluminum base shoes. The laboratory faucet has a swinging gooseneck spout and hose connection. A real convenience is the flask washer. The flask opening is placed over a rubber protected water jet. Then the flask is pushed to rear against a rubber cradle and water jet completely sprays flask. The 6-foot hose shown is equipped with a grip valve and cut-off valve. This hose makes it easy to add cooling water to flasks on a cart. This unit may be purchased complete as described above (Model C-452-C) or individual components may be purchased separately.

LABCONCO

Laboratory Construction Company

8811 Prospect Avenue — Kansas City, Missouri

CEREAL CHEMISTRY

VOL. 37 SEPTEMBER, 1960 NO. 5

THE SUBSIEVE-SIZE FRACTIONS OF A HARD RED SPRING WHEAT FLOUR PRODUCED BY AIR CLASSIFICATION[1]

REZSOE GRACZA[2]

ABSTRACT

A conventionally milled hard red spring wheat flour was fractionated by repeated air classifications.

The repetitive procedure, starting with separation at low particle size, produced fractions with protein contents as high as 20.3% and as low as 8.8% from a parent flour of 13.8% protein content and 86% extraction (flour basis) of a 100% Montana hard spring wheat.

The degree of protein shifting index shows that 5.4% of the parent flour's 13.8% protein could be shifted within the fractions, compared to 27.7% protein shifting in the identically processed 7.8% protein content soft red winter wheat flour described in a previous paper.

Other indices such as ash, diastatic activity, alkaline water retention capacity, thiamine, fat, and MacMichael viscosity appreciably increased with increasing protein content and/or specific surface within the subsieve size range. Bulk density increased with increasing particle size, whereas specific gravity decreased with increasing protein content in the fractions. The pH value reached its maximum in the fraction of lowest protein content.

Physical dough test data indicate that the dough development time increased with increasing protein content. Absorption, however, increased not only with the protein content but also with decreasing particle size, i.e., increasing specific surface. Doughs made from flour particles collected in the 44.5 to 48.0 SED micron size range yielded extensigrams characteristic of good bread flours, whereas doughs made from flour particles collected in the 23.0 to 39.0 SED micron size range produced farinograms and extensigrams characteristic of soft wheat flours used in pastries.

This paper reports the response of a hard red spring wheat flour to a repeated air-classification procedure described in a previous paper (8) which showed the response of a soft red winter wheat flour to the same treatment. The use of identical procedures and about the same analytical indices facilitates a comparison of the behavior of hard and soft wheat flours in air classification.

In the former paper (8), the literature review was restricted to size

[1] Manuscript received October 3, 1959.
[2] Address: The Pillsbury Company, Minneapolis, Minnesota.

reduction and size classification studies closely related to the subject matter of the paper. Since, in a broader sense, histological observations and protein isolation from flour are related to the subject matter, some extension of the references of the previous article is here offered.

Histological studies of the wheat, and especially of the endosperm, are probably as old as the microscope itself. At the turn of the century, Cobb (6) formulated the obvious observation that "the starch granules in each flour-cell of the ripe (wheat) grain are held enmeshed in the dry and tough elaborate network of protoplasmic matter which, when soaked, assembled, and weighed by the chemist, is entered in his returns under the name of gluten." He further stated that "the tendency of the starch grains (granules) during the process of making flour is to fly loose." On the basis of this observation, Cobb was able to shift some protein in a flour obtained from the innermost section of the wheat kernel, using a 112-mesh "fine silk" sieve. He is probably the first to record protein shifting by recognizing the heterogeneity of the wheat endosperm. Greer *et al.* (9) elaborated, in their morphological studies, on the different shapes and sizes of the wheat endosperm fragments with particular reference to the locational origin of fragments and cell walls attached to them. This morphological study was continued by Kent and Jones (14) with particular reference to distinguishing between the endosperm fragments of break and reduction flours.

Hess and Hanssen (13) described a procedure for the production of native vegetable protein (and cereal protein) using density separation in nonaqueous liquids advantageously selected to float the protein material while the heavier constituents of the flour could be separately collected. Their work was aimed at producing protein in a native form, as contrasted to the commercially washed gluten obtained by methods first described by Beccari (5). Studying flour and protein matrix obtained by density separation, Hess observed that individual starch granules and some protein particles were freed during milling (11). Hanssen *et al.* (10) measured the size distribution of different cereal and leguminous starches using a microscope. They found that starches in the wheat flour range in diameter from 0.55 to 55 microns and the "protein lamellae" obtained by density separation have a thickness of 0.26 micron. The latter measurement was based on the average surface area of protein particles (50 square microns) as measured by microscopic observation.

These studies reveal that the concentration or isolation of proteinaceous substances from cereal flours may be obtained by different means such as 1) by washing out the starch granules with the use of aqueous media (5); 2) by flotation in nonaqueous liquid media using

density differences between the protein and the starch granules (12); and 3) by flowdynamic classification utilizing simultaneously the size, density, and shape of the partly homogeneous and partly heterogeneous flour particles (8).

Applying different principles, different degrees of protein concentration and/or isolation are obtained. Owing to the different principles involved, the protein and starch products obtained by the different methods exhibit differences. Such differences were recorded in detail by Hess (12) in comparing water-washed gluten with protein material obtained by density separation.

Materials and Methods

The parent flour was an unbleached, untreated 86% extraction (flour basis) patent flour milled commercially from 100% Montana hard red spring wheat having 16% protein content and 60.0 lb. per bushel test weight measured at 12.1% moisture. This parent flour was fractionated, without additional grinding, 4 months later. Thus, the separations can be considered typical of what may be obtained from spring wheat flours by this classification procedure.

This parent flour was fractionated with process variables identical to those used for the soft wheat flour fractionation (8) in a commercial-size air-classifier which was provided with a special, forced vortex type rotor in the classification zone similar to the type described by Lykken (15). This fractionation produced seven subsieve-size fractions from the parent hard wheat flour.

The flowsheet (Fig. 1) identifies the samples with capital letters and indicates the critical-cut size of the classification obtained by analysis of the particle size distribution curves of the fine and coarse flour fractions described in a previous paper (8). The parent stock was subjected to a first-stage air separation at 21 SED micron critical cut resulting in a fine fraction, A, and in a coarse fraction, AA. The first-stage coarse fraction, AA, was subsequently subjected to a second-stage air separation at a larger critical cut (23 SED micron), resulting in a fine fraction, B, and in a coarse fraction, BB. The classification of the coarse fractions was repeated five times, so that the total procedure resulted in seven fractions, namely A, B, C, D, E, F, and FF.

The description of the critical cut and sharpness figures and their interpretations are described in the previous paper (8). The method used to obtain the particle size data was a slight modification of the method described by Whitby (16).

The average particle diameter figures were obtained by the Fisher

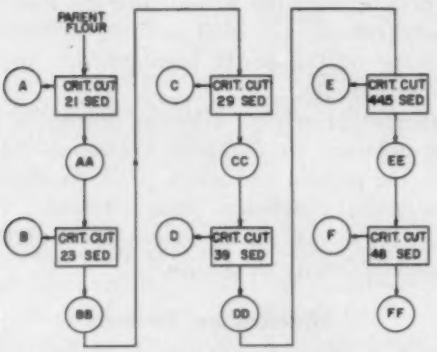


Fig. 1. Flow diagram of the repetitive classification procedure with increasing critical-cut sizes.

subsieve-sizer apparatus (7) which uses the principle of permeability through a porous bed.

The following analyses were made with slight modifications of the procedures described in *Cereal Laboratory Methods* and identified by their test numbers (1): protein 67.1, moisture 48.3, ash 9.1, diastatic activity 24.1, pH 60.2, thiamine 98.8c, and MacMichael viscosity 97.1. Specific gravity was determined by ASTM Method C-188 (3), petroleum ether-extractable fat by AOAC Method 22.26 (4), and free fatty acid content by AOCS Method CA5a-40 (2). The two last-mentioned methods were modified slightly. Alkaline water retention capacity was obtained by the method of Yamazaki (17). Color reflectance values were obtained from a kerosene-flour slurry measured on glass with a Gardner Differential Tristimulus colorimeter. Bulk density data were obtained by the method described in the previous paper (8).

Farinograms were obtained using the 300-g. mixer and the Constant Flour Method (Method 26.4). The extensigrams were determined by the procedure described previously (8), except that all samples were tested by the longer remix option and the 3-hour schedule normally used for bread flours. The scale resistance was set at 2:1; i.e., 1,000 g. resistance registers 500 B.u. Resistance to extensibility was measured at 500 mm. deformation uncorrected for cradle travel.

Results and Discussion

Microscopic Studies. Figures 2 and 3 are photomicrographs of the

hard spring wheat parent flour and its seven subsieve-size fractions using 180× magnification.

Comparison with the photomicrographs of the soft wheat flour fractions (8) reveals the same trend in the proportion of protein matter to starch granules that was observed in the soft wheat flour fractions. The following differences are evident:

a. The elementary protein matter particles appear to be smaller, thinner, and less irregular in shape than those from the soft wheat flour. This is evident in the A and B fractions.
b. The elementary starch granule particles generally appear to have a flatter lenticular shape than those from soft wheat flour. These flatter granules split into halves or into sectors of an ovoid shape

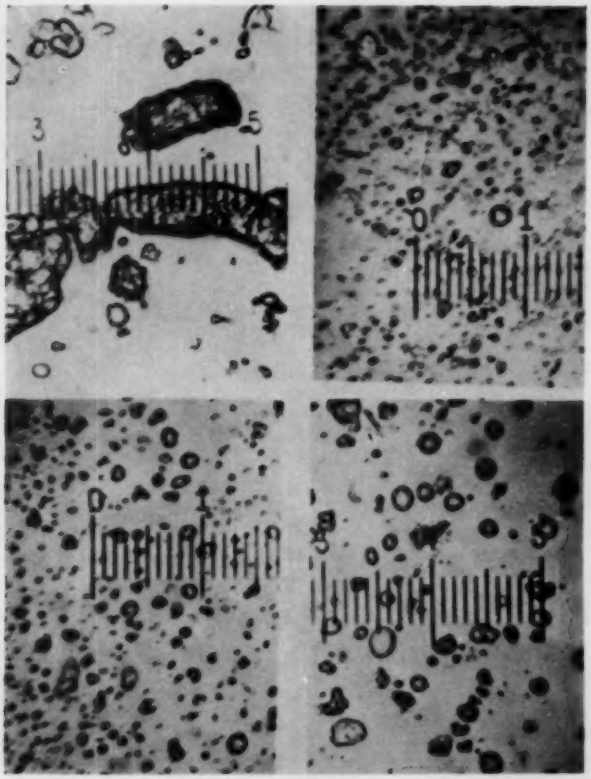


Fig. 2. Photomicrographs of the parent flour (upper left), the A fraction (upper right), the B fraction (lower left), and the C fraction (lower right). All photographs were taken at 180× magnification. Slides prepared in an oil of 1.600 refractive index.

more frequently than those from a soft wheat flour as seen in the C, D, and E fractions.

c. The surfaces of the individual starch granules or starch granules in smaller aggregates do not appear to have been stripped from the protein matrix portions as cleanly as those originating from soft wheat flour. This is evident in all fractions to different degrees except the last fraction (FF).

d. The number of large elementary starch granules with or without some protein matrix on their surface is considerably smaller than that observed in a soft wheat flour; note the photograph of the parent stock.

e. Endosperm chunk particles have polygonal shapes with definite edges, a large portion of the cells being similar in shape to a par-

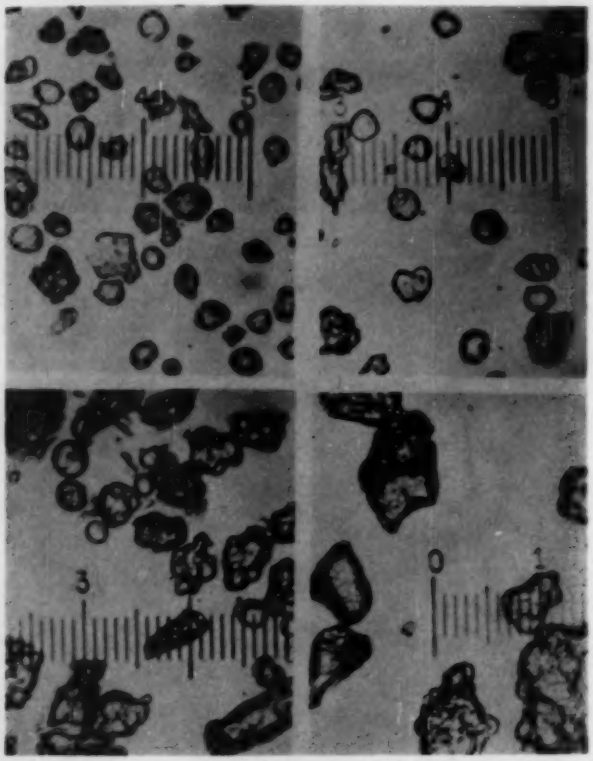


Fig. 3. Photomicrographs of fractions D (upper left), fraction E (upper right), fraction F (lower left), and the residual fraction FF (lower right). All photographs were taken at 180× magnification. Slides prepared in an oil of 1.600 refractive index.

allelepiped. Soft wheat flour endosperm chunk particles, in comparison, have a rounded-off shape and smeared-off, furry edges with starch granules occasionally protruding from the body of the particle. See parent and FF fractions.

f. Many of the endosperm chunk particles contain large fissures, a bodily discontinuation which is practically absent from chunk particles of soft wheat origin.

Protein Content of the Fractions. The protein histogram, Fig. 4, illustrates that the classification steps resulted in a concentration of

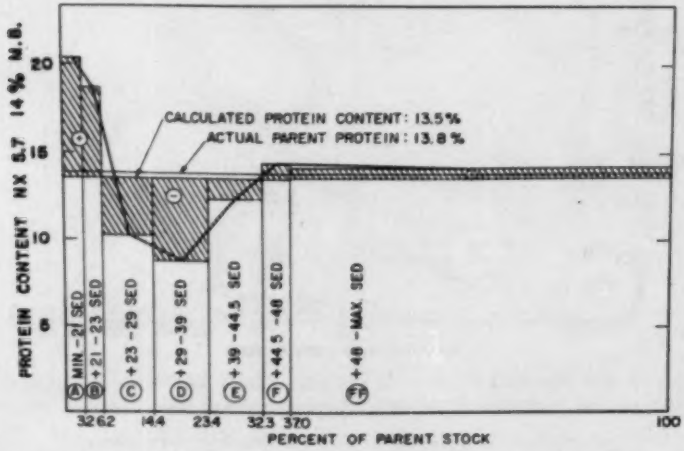


Fig. 4. Histogram of protein content versus percent of parent stock from the seven subsieve-size fractions of a hard red spring wheat flour, illustrating the positive and negative protein shifting in the respective fractions.

protein in fractions A, B, F, and FF, while fractions C, D, and E were depleted of protein as compared to the distribution of protein and starch in the parent flour.

Utilizing the term *degree of protein shifting*[3] employed in the previous paper, a comparison can be drawn between the response of the soft red winter wheat flour and that of the hard red spring wheat flour. It was possible to shift 5.4% of the hard wheat flour's protein content within the seven fractions, whereas 27.7% of the soft wheat flour's protein was shifted in an identical classification procedure.

[3] Degree of protein shifting expresses the percentage of the protein content of the original flour which was shifted into or out of the specific fractions as a result of air classification. Of necessity, the portion of protein which was shifted out of the low-protein fractions has to be found in the high-protein fractions (8).

From a fitted curve connecting the midpoints of the bars, or by equating areas under and above the curve, it is possible to estimate yields at a chosen protein content. Another useful form of such a histogram is made by plotting the midpoints of the protein content as a function of particle size. This permits the estimation of the protein content per size interval or the protein content versus particle size if infinitesimally small size intervals are used.

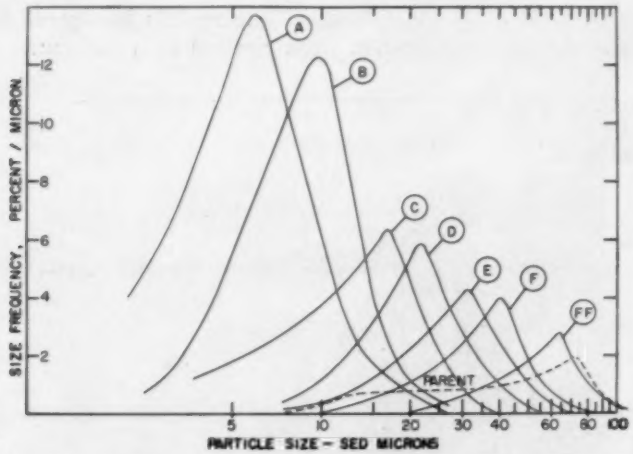


Fig. 5. Size distribution curves of the parent flour and its seven subsieve-size fractions plotted as percent per micron versus logarithm of size.

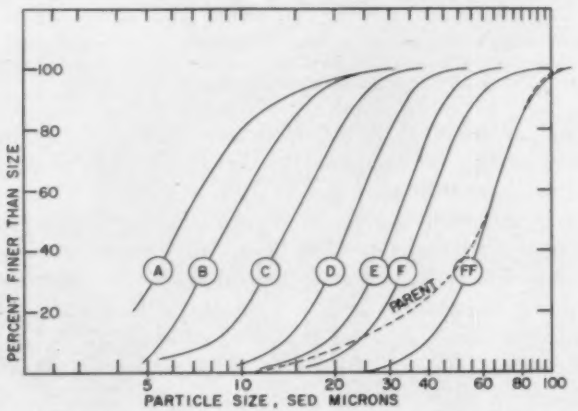


Fig. 6. Cumulative size distribution curves of the parent flour and its seven subsieve-size fractions. Capital letters A, B, C, D, E, F, and FF designate increasingly coarser fractions.

Particle Size Data. The particle size distribution curves of the parent flour and its fractions are presented in Figs. 5 and 6, where the same distribution functions are plotted in percent/micron versus log SED size, and percent finer than size versus log SED size. These curve families are indicative of the closeness of the critical-cut size ranges of the fractions.

Table I presents the numerical data for particle size distribution as obtained by the slightly modified Whitby centrifuge technique (16).

TABLE I

PARTICLE SIZE DATA OF PARENT FLOUR AND ITS SEVEN SUBSIEVE-SIZE FRACTIONS, OBTAINED BY THE MODIFIED WHITBY SEDIMENTATION METHOD

SED Microns	Percent Finer than Size of the Parent Flour and Fractions							
	Parent	A[a]	B	C	D	E	F	FF
120	100.0	....	....	....	....	....	....	100.0
100	98.0	....	....	....	....	....	100.0	97.1
80	85.6	....	....	....	....	100.0	98.8	86.8
60	47.7	....	....	....	100.0	98.9	92.7	46.6
40	23.8	....	100.0	100.0	97.2	79.0	55.1	10.5
30	15.5	100.0	98.7	97.5	79.7	44.2	23.0	2.7
20	7.7	97.2	94.7	75.7	35.3	12.5	6.6	0.0
10	0.5	80.4	55.3	18.0	2.3	0.6	2.4	0.0
5	0.0	27.0	4.0	4.4	0.0	0.0	0.0	0.0

[a] The sequence of the separation is such that the size increases from A through FF. A through F are the fine fractions of the six process steps, FF being the coarse fraction of the last step.

A cumulative curve of the protein content of the fine and coarse fractions respectively, plotted against critical-cut size, is shown on Fig. 7. The two converging curves meet at the neutral critical cut (8) 31.3 SED micron. The curve pair may be useful as it provides an approximation of the protein content of the coarse and fine products if the parent stock is processed at an arbitrary critical cut as shown on the abscissa.

Other Analytical Indices. The results of other analyses carried out on the parent flour and the subsieve-size fractions are recorded in Table II.

The moisture content in the fractions decreased with smaller average particle size (Fisher); a greater moisture loss during air classification would naturally be expected in those fractions which possess high specific surfaces. Ash content is generally higher in the fine fractions and apparently varies with the protein content of the fraction, except in the coarsest fraction, DD.

Diastatic activity values, when compared to a similar separation of soft wheat flour, are considerably higher though they follow a similar pattern. The higher diastatic activity values are evident where cleaved

TABLE II

Analytical Data for Parent Flour and Seven Subsieve-Size Fractions of a Montana Hard Spring Wheat Flour

Product	Yield as Percent of Parent	Critical Cut Size Range, SED Micron	Sharpness	Av. Particle Size (Fisher)	Protein[a] N × 5.7	Moisture	Ash	Diastatic Activity, mg. Maltose per 10 g.	AWRC[b]	pH	Specific Gravity	Bulk Density	Color Reflectance Rd by Hunter	Thiamine	Viscosity	Petroleum Ether-Extractable Fat	Free Fatty Acid Fat Basis
			%	μ	%	%	%		%		g/cm³	g/cm³		mg/lb	°MacM	%	%
Parent	100.0	min.-max.	..	23.2	13.8	11.4	0.400	258	74.2	5.82	1.447	0.612	53.7	0.39	197	1.06	15.1
A	3.2	min.-21	82	4.3	20.3	8.9	0.742	528	138.0	5.90	1.430	0.263	58.5	1.96	+300	2.09	24.9
B	3.0	21-23	81	5.2	18.7	8.8	0.749	490	130.0	5.92	1.431	0.294	60.0	1.53	219	1.84	21.7
C	8.2	23-29	79	9.6	10.3	9.5	0.504	523	109.0	5.98	1.458	0.440	61.7	0.61	110	1.04	20.2
D	9.0	29-39	76	14.6	8.8	10.8	0.443	379	67.7	6.00	1.465	0.567	60.4	0.48	56	0.89	18.0
E	8.9	39-44.5	71	17.0	12.4	10.9	0.484	282	70.8	6.02	1.458	0.616	55.2	0.48	131	1.04	17.3
F	4.7	44.5-48	61	19.5	14.5	10.9	0.455	213	71.2	5.95	1.451	0.641	52.3	0.48	182	1.10	16.4
FF	63.0	48-max.	..	30.0	14.2	10.7	0.325	129	63.5	5.73	1.450	0.762	53.0	0.22	146	0.97	14.4
AA	96.8	+21-max.	..	24.0	13.3	11.3	0.394	237	68.7								
BB	93.8	+23-max.	..	24.2	13.0	11.0	0.375	214	66.6								
CC	85.6	+29-max.	..	26.2	13.6	10.5	0.350	187	63.3								
DD	76.6	+39-max.	..	28.0	14.2	10.7	0.354	161	63.3								
EE	67.7	+44.5-max.	..	29.8	14.3	10.7	0.333	141	60.5								

[a] Protein and other test results are expressed on a 14% moisture basis.
[b] Alkaline water retention capacity.

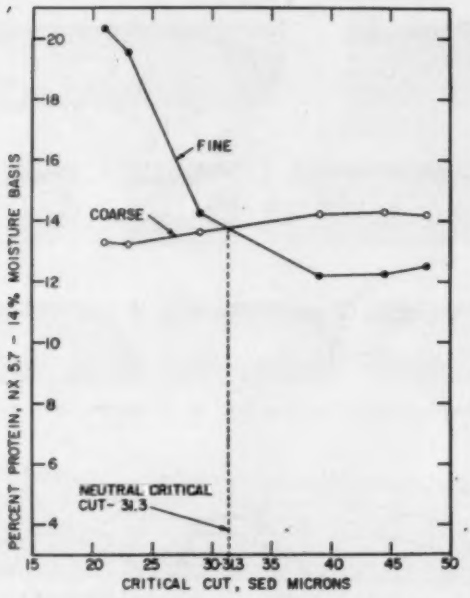


Fig. 7. Protein content of the fine and coarse fractions plotted as functions of critical cut, illustrating the neutral critical cut for a commercial hard spring wheat flour.

starch granules and/or the enzyme accumulate, i.e. in the lower size groups. Alkaline water retention capacity distribution in the fractions, as with soft wheat flour separations, follows primarily the specific surface and/or the protein content and perhaps other components.

The free fatty acid portion of the fats, as shown by the free fatty acid content expressed on a fat basis, increases in the fractions with decreasing particle size.

The specific gravity of the fractions increases with a decrease in protein content, a trend which was also observed in the fractionation of soft wheat flour (8).

Physical Dough Test Data. The farinograms and extensigrams (Figs. 8, 9; Table III) for doughs made from the various fractions show the same general trends observed in doughs made from sofe wheat fractions (8). Dough development times increase with the protein content. Absorption, however, does not increase directly with protein content but varies also with the surface characteristics of the fractions; with higher specific surface, the absorption increases. The major changes in

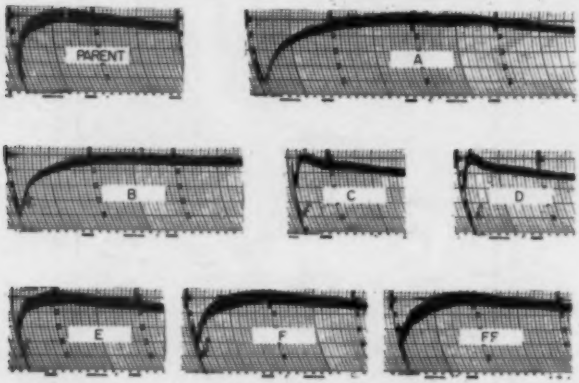


Fig. 8. Farinograph curve characteristics of the parent flour and the seven subsieve-size fractions.

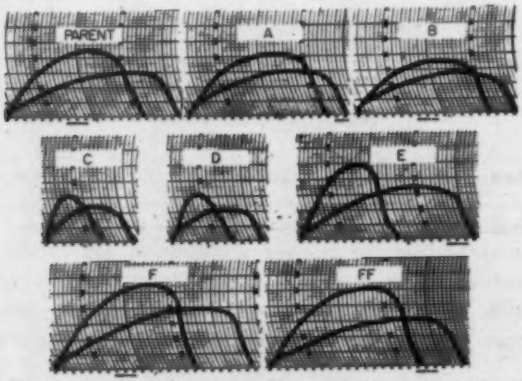


Fig. 9. Extensigraph curves of the parent flour and the seven subsieve-size fractions.

the dough-handling properties are illustrated by the area under the 1-hour extensigraph curves; the area increases about threefold as the protein increases from the lowest to the highest values. This compares to a tenfold change in the fractions of a soft wheat flour (8), indicating also the poorer protein-shifting possibilities in hard spring wheat flours. The arrival time plus dough development time in the finest fractions (A, B) of the hard wheat flour is about half as long as that in the soft wheat flour, indicating an increased rate of hydration at approximately a comparative protein level and a somewhat higher absorption.

While the highest extensibility and/or resistance desired for bread-baking is exhibited in the finest fractions of a soft wheat flour

TABLE III

MAJOR INDICES FROM THE FARINOGRAPH AND EXTENSIGRAPH TESTS OF A HARD RED SPRING WHEAT FLOUR AND ITS SEVEN SUBSIEVE-SIZE FRACTIONS

PRODUCT	ABSORPTION	FARINOGRAPH					EXTENSIGRAPH					
		Dimension[a]				Valorimeter	One Hour			Three Hours		
		A	B	C	D		Extensibility	Resistance	Area	Extensibility	Resistance	Area
	%						mm	B.u.	cm²	mm	B.u.	cm²
Parent	64.7	3.50	6.50	9	30	64	198	155	76	157	285	89
A	89.7	12.50	20	17.25	5	95	181	180	71	160	270	86
B	89.9	8	16	17.50	10	91	181	170	64	152	240	73
C	72.6	1.25	2	1.75	50	41	98	220	32	65	300	23
D	63.7	1.25	1.50	1.25	95	34	105	190	30	70	315	28
E	62.6	2	5.25	8.75	30	59	199	170	86	113	365	66
F	66.9	5.25	9	10.75	30	73	228	170	110	163	320	114
FF	64.4	4.75	9	11.50	30	73	226	145	87	175	280	107

[a] A, time in minutes from the start of water addition until the top of the curve intercepts the 500-B.u. line, "arrival time." B, C, and D, respectively, equivalent to Dough Development Time, Stability, and Tolerance Index (*Cereal Lab. Methods*, 6th ed., sec. 26.4 (see ref. 1)).

from the lower end of the wheat hardness spectrum (8), the same can be observed in the coarsest fractions of hard spring wheat flour from the highest end of the wheat hardness spectrum. The particles collected in the F fraction produced dough development properties optimum for bread-baking. In economic considerations the removal of a portion of the parent flour, such as fractions C and D with poor extensibility, will enhance desired flour properties in bread-baking. The farinograph and extensigraph characters of the C and D fractions are practically identical to those desired in a pastry flour milled from soft wheat.

Thiamine content generally preponderates in the high-protein fractions, though the over-all trend does not follow the protein content but rather the specific surface.

The bulk density data show a general decrease with increasing specific surface.

Soft wheat flour is inherently low in protein content but more responsive to air classification. It is adaptable to the withdrawal of a high-protein fraction leaving fractions applicable to cake flours and low-protein or starchlike applications.

Hard wheat flour, however, shows protein shifting to a lesser degree but the resulting fractions offer a different set of products. Fractions with protein contents and properties may be produced which cover the desirable properties of both pastry- and bread-baking. Thus, a great amount of latitude is available within which properties of flour may be altered by air classification or blending.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. American Oil Chemists' Society. Official and tentative methods (2nd ed.); CA5a-40. The Association: Chicago, Illinois (1946).
3. American Society for Testing Materials. ASTM Standards, 1946, Part II, p. 78 (1946).
4. Association of Official Agricultural Chemists. Official methods of analysis (8th ed.), p. 371. The Association: Washington, D.C. (1955).
5. Beccari. De Bononiensi Scientiarum et Artium Instituto Atque Academia Comentarii. Vol. II, Part 1, p. 122 (1745). (Excerpted by A. Maurizio in: Die Nahrungsmittel aus Getreide, Vol. I, Part 8, p. 404. Paul Parey: Berlin (1917).)
6. Cobb, N. A. Universal nomenclature of wheat. Agr. Gaz. N. S. Wales, Misc. Pub. No. 539, Dept. of Agriculture, New South Wales (1905).
7. Gooden, E. L. Air permeation apparatus for measuring the coarseness of powders. U. S. Patent No. 2,261,802 (November 4, 1941).
8. Gracza, Rezsoe. The subsieve-size fractions of a soft wheat flour. Cereal Chem. **36**: 465–487 (1959).
9. Greer, E. N., Hinton, J. J. C., Jones, C. R., and Kent, N. L. The occurrence of endosperm cells in wheat flour. Cereal Chem. **28**: 58–67 (1951).
10. Hanssen, E., Dodt, Erika, and Niemann, E. G. Bestimmung von Korngrösse Kornoberfläche, und Korngewicht bei pflanzlichen Stärken. Kolloid-Z. **130**: 19–31 (1953).

11. Hess, K. Müllerei und Forschung. Die Mühle **89:** 428–431 (1952).
12. Hess, K. Changes in the protein and starch of wheat flour during mixing and baking. Trans. Am. Assoc. Cereal Chemists **11:** 153–166 (1953).
13. Hess, K., and Hanssen, E. Verfahren zur Gewinnung von nativem, pflanzlichem Eiweiss. German Patent No. 960,331 (March 21, 1957).
14. Kent, N. L., and Jones, C. R. The cellular structure of wheat flour. Cereal Chem. **29:** 383–398 (1952).
15. Lykken, H. G. Centripetal classifier. U. S. Patent 2,741,336 (April 10, 1956).
16. Whitby, K. T. A rapid general purpose centrifuge sedimentation method for measurement of size distribution of small particles. Part I. Apparatus and method. Heating, Piping and Air Conditioning **27:** 231–237 (January 1955); Part II. Procedures and applications, pp. 139–145 (June 1955).
17. Yamazaki, W. T. An alkaline water retention capacity test for the evaluation of cookie baking potentialities of soft winter wheat flours. Cereal Chem. **30:** 242–246 (1953).

MILLING PROPERTIES OF WHEAT IN RELATION TO PEARLING, SCOURING, AND IMPACTION[1]

G. M. Grosh, J. A. Shellenberger, and E. P. Farrell

ABSTRACT

Pearled hard red winter wheat when experimentally milled gave a higher-ash flour than unpearled wheat. It was possible to effect relatively clean removal of the outer pericarp from the wheat kernel by scouring, but this peeling adversely affected subsequent milling results. Samples of Ponca wheat were tempered to 14 or 16.5% or untempered, allowed to stand for 0, 4, 12, or 24 hours, and impacted on a 27-in. Entoleter at 1,600, 2,000, or 2,400 r.p.m. It was possible with both Ponca seed and a commercial hard winter wheat to effect a 0.028% decrease (7 to 9% of the flour ash) in ash content of 60% extraction flour by prebreak impaction of tempered wheat just prior to milling, using a 27-in.-diameter impact machine at 2,400 r.p.m. Prebreak impaction studies with soft wheat indicated no significant improvement in experimental milling results.

While numerous articles have appeared in the trade journals on the use of abrasive or peeling techniques to remove the bran and germ prior to milling (3,4,5,12,13,14,18), few data were offered verifying any benefit to milling results. During the 1920's, some milling companies in this country adopted peeling systems only to abandon them later. In Europe a few mills are said to use peeling or severe scouring prior to milling.

[1] Manuscript received August 3, 1959. Contribution No. 321, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Kansas State University, Manhattan. A report of work done under contract with USDA and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Northern Utilization Research and Development Division of Agricultural Research Service, Peoria, Illinois.

Impact machines are used widely in the milling industry to kill and remove insects before the wheat is milled. Anderson (2) reported in a study of premilling impaction at lower impact velocities that this had no effect on milling behavior of wheat. Since the nature of impact grinding (10) is different from that of grinding by rolls, it is conceivable that a moderate reduction by prebreak impaction might improve the milling behavior of wheat by lowering the ash content of the flour at a given extraction.

These studies were undertaken to determine if various physical treatments of wheat prior to milling might benefit the milling properties of hard and soft wheats.

Materials and Methods

A hard red winter wheat, variety Ponca, with 12.6% protein and 1.64% ash (14% moisture basis) was used in the majority of the studies reported. A commercial hard winter wheat mix with 11.5% protein and 1.77% ash and a soft red winter wheat, variety Brevor, with 10.7% protein and 1.78% ash were also used.

Pearling. A Strong-Scott laboratory barley pearler driven by a 1/6 h.p., 1,725 r.p.m. motor was used to observe the value of abrasive action as a prebreak treatment. Wheat was tempered to 16% moisture 24 hours before pearling and experimental milling. To 2 kg. tempered wheat, 0, 2, or 5% water was added and the material was mixed for 10 minutes. Samples were pearled 0, 20, 40, or 60 seconds. The wheat sample (100 g.) was placed in the hopper of the pearler, with the outlet blocked, and abraded from 0 to 60 seconds. Polishings removed were determined by sifting the stock on a 14-mesh screen in a Ro-Tap sifter for 2 minutes.

Scouring. A Forster Model 10 wheat scourer driven by a ½ h.p., 1,725 r.p.m. motor was modified by installing paddles the length of the scouring chamber and closing the outlets to permit more vigorous scouring action for varying times up to 1 minute. To observe the effect of scouring on milling behavior, samples of Ponca wheat were tempered to 14.5, 15.5, and 16.5% moisture, allowed to stand 24 hours, wetted with an additional 0, 3, or 5% water, mixed for 5 minutes, scoured 0, ½, or 1 minute, and then milled.

Impaction. An Entoleter with 27-in.-diameter rotor with pin rotor and smooth stator liner was used in the studies reported in this paper. Wheat was impacted at 1,600, 2,000, or 2,400 r.p.m. under seven representative tempering conditions as indicated in Figs. 3 and 4. Time between initial wetting and milling was always 24 hours; moisture content at time of milling was always 16.5%, adjusting to

that moisture level by an additional wetting after impaction. Each experiment was replicated a minimum of four times. The four prebreak impaction treatments which showed most improvement of milling behavior (reduction of ash content at constant flour yield) as indicated in Table II, when compared to the unimpacted control sample, were repeated in experimental milling trials ten times. Mean values of the product of ash and percentage of product of each flour stream from each treatment were calculated (corrected to 14% moisture basis), and cumulative ash-extraction curves were plotted.

Sectioning and Hardness Test. Plane kernel slices were prepared with a freezing microtome sectioning technique employed in this laboratory (9). Hardness tests on 15 kernels at each treatment were determined by means of the MIAG hardness tester (16).

Analyses. Ash, protein, and moisture determinations were performed by using procedures 9.1a, 67.1, and 48.3a as outlined in *Cereal Laboratory Methods* (1).

Experimental Milling. There is no agreement on a standard experimental milling procedure. Because of the many factors which can affect results (8,19), it is difficult to reproduce such millings with accuracy, even under carefully controlled conditions. Geddes and West (7) were able to minimize variations in yield to 1% of the total product, but careful roll adjustment, temperature, and humidity control were essential. By using the Allis-Chalmers experimental milling equipment, it was possible to minimize variations in results within each day, but variations from day to day were more difficult to control. For this reason, the randomized block experimental design was used, in which each treatment was compared with a control sample milled on the same day, permitting allowance for possible day-to-day variations when results were analyzed.

The experimental milling flowsheet (Fig. 1) was used in most of the studies reported. Earlier studies concerned with pearling, scouring, and prebreak impaction used a flow similar to that illustrated but with minor alterations of sieve sizes and break corrugations. Break roll corrugations were as follows: first and second breaks, 7 in. by 7 in., 14 corrugations per inch, ½-in. spiral, 2:1 differential dull to dull; third and fourth breaks, 24 corrugations per in., ½-in. spiral, 2:1 differential dull to dull. Roll spacings were: first break, 0.025 in.; second break, 0.016 in.; third break, 0.006 in.; fourth break, 0.004 in.; first sizings, 0.003 in.; second sizings, 0.002 in. Smooth rolls were adjusted to remove 30% of the control stock to the rolls to pass through 11xx silk screen. Sifting times were standardized as follows:

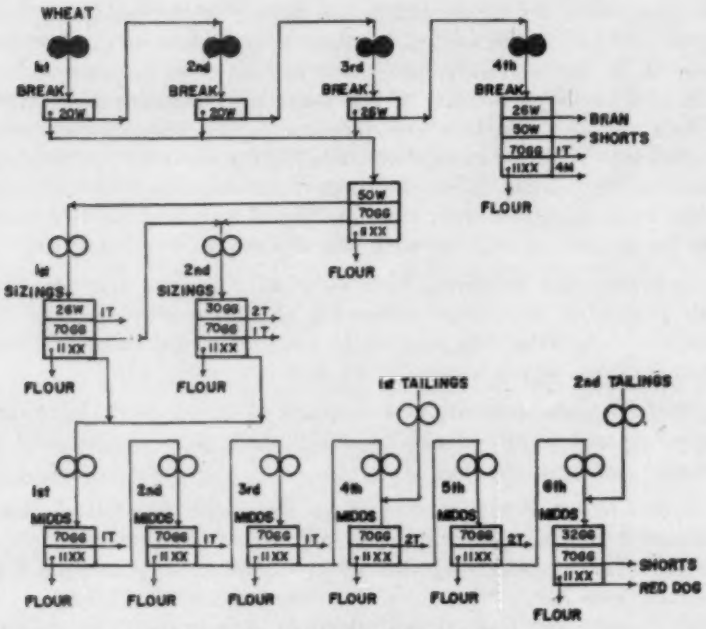


Fig. 1. Experimental milling flowsheet.

breaks, 1 minute; sizings and low-grade middlings, 1.5 minutes; first to third middlings, 2 minutes.

Seeborg (15) proposed an experimental milling score recognizing five interdependent factors contributing to milling quality: milling rate, optimum milling moisture, total flour yield, patent flour yield, and ash of patent and straight-grade flour. Flechsig (6) also developed a standard milling technique which comprises power consumption, flour ash at constant yield, and optimum moisture level. For the purpose of these studies where moisture and wheat sample were constant throughout the experiment, it was desirable to have a single quantitative index of milling quality, and with flour ash content and yield interdependent, the cumulative ash curve was plotted for each sample milled. The flour produced from each sample was removed as five composite streams (a, break; b, sizings; c, first and second middlings; d, third to fifth middlings; e, sixth middlings and tailings), weighed, sampled, and analyzed for ash and moisture content. After correction of weight and ash value to a 14% moisture basis, curves which showed a change in ash content with increasing extraction were plotted. From each curve it was possible to obtain

a single ash value at constant yield as a criterion of milling quality.

Baking Test. The straight dough procedure as outlined in *Cereal Laboratory Methods* (1) using the following bread formula was followed: flour, 700 g.; sugar, 35 g.; salt, 14 g.; shortening, 21 g.; Arkady, 3.5 g.; malt, 3.5 g.; nonfat dry milk, 21 g.; yeast, 14 g.; water, based on farinograph absorption.

Results and Discussion

Pearling Studies. A typical set of analytical results is presented in Table I. Ash content of the wheat decreased and protein content of the polishings increased as the quantity of polishings removed

TABLE I

EFFECT OF VARIOUS PEARLING TREATMENTS PRIOR TO MILLING ON POLISHINGS REMOVED AND EXPERIMENTAL MILLING BEHAVIOR

Moisture Added	Pearling Time	Polishings			Wheat		Flour Yield[a]	Ash Content at 70% Extraction
		Yield[a]	Ash	Protein	Ash	Protein		
	seconds	%	%	%	%	%	%	%
Control	..	0	..	...	1.7	12.7	76.1	0.46
2	40	6.7	4.7	14.4	1.5	12.8	70.4	0.53
5	40	5.5	4.4	12.8	1.6	13.2	74.4	0.50
5	60	10.9	4.4	15.8	1.3	12.5	73.6	0.52

[a] Expressed as percent of total product including polishings.

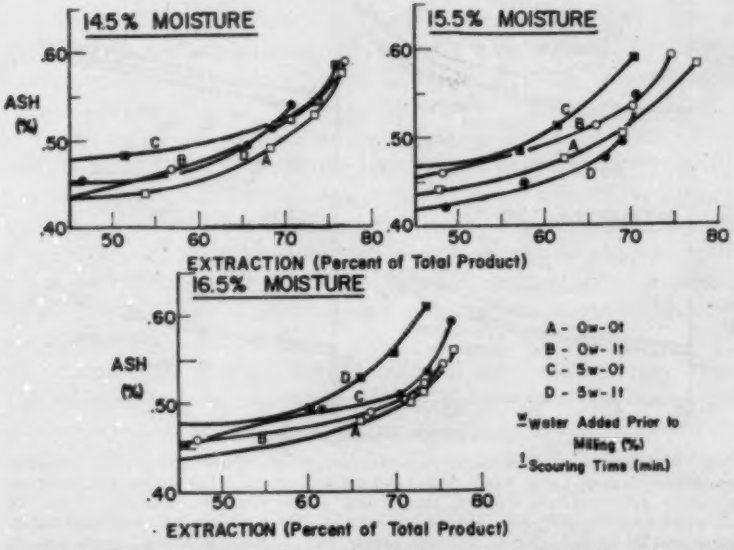


Fig. 2. Effect of wetting and scouring of wheat tempered to 14.5, 15.5, or 16.5% moisture on cumulative ash-extraction curves.

increased with longer pearling time. This may be due to removal with the polishings of the aleurone layer, which is high in both ash (11) and protein content. In no instance, on repetition of these studies, did pearling improve milling behavior as evidenced by flour yield or 70% extraction ash content.

Scouring Studies. Preliminary trials indicated that it was possible to obtain a visibly clean separation of the outer pericarp from the seed coat when 5% water was added 5 minutes before scouring. Temperature (from 20° to 100°C.) of the water did not affect the percentage of scourings removed. Use of more than 5% water before scouring did not appreciably increase the percentage of outer pericarp (wing) removed.

Cumulative ash-extraction curves (Fig. 2) showed that in most cases observed, scouring, with or without the addition of moisture, increased the ash content of the flour at the same yield as flour from unscoured wheat. It is possible that scouring damages the bran surface and this increases bran contamination and, hence, ash content of the flour produced.

Prebreak Impaction Studies. Preliminary experiments using a 10.5-

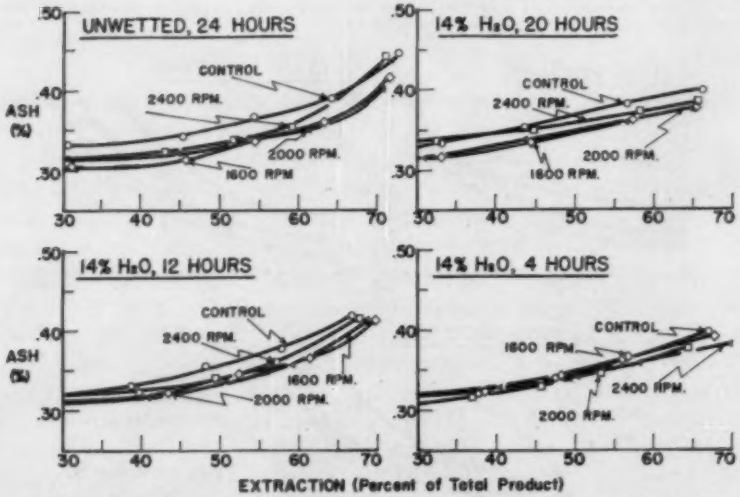


Fig. 3. Effect of various impaction treatments of unwetted or 14% moisture Ponca Seed wheat at 1,600, 2,000, and 2,400 r.p.m. with 27-in.-diameter Entoleter on cumulative ash-extraction curves. Upper left: Impaction of unwetted wheat 24 hours prior to milling. Upper right: Impaction at 14% moisture, 4 hours after initial wetting and 20 hours prior to milling. Lower left: Impaction at 14% moisture, 12 hours after initial wetting and 12 hours prior to milling. Lower right: Impaction at 14% moisture, 20 hours after initial wetting and 4 hours prior to milling.

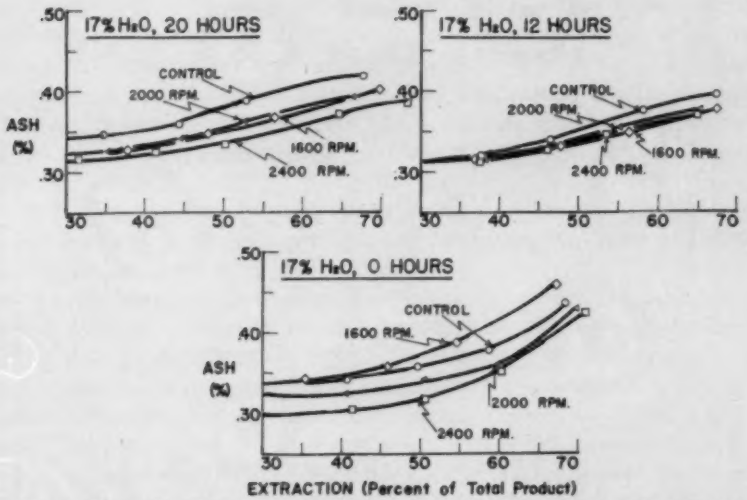


Fig. 4. Effect of various impaction treatments at 16.5% moisture of Ponca Seed wheat at 1,600, 2,000, and 2,400 r.p.m. with 27-in.-diameter Entoleter on cumulative ash-extraction curves. Upper left: Impaction 4 hours after initial wetting and 20 hours prior to milling. Upper right: Impaction 12 hours after initial wetting and 12 hours prior to milling. Bottom: Impaction 24 hours after initial wetting, immediately before milling.

in.-diameter Entoleter showed that a decrease in ash content as much as 0.09% at the same extraction (i.e., decrease from 0.51 to 0.42%) could result from prebreak impaction. When plane sections of impacted and unimpacted wheat kernels were prepared prior to milling, it was noted that as impaction speed increased and moisture content decreased, the regular radial and transverse cracking pattern, which can be noted in hard wheats as a result of tempering (9), was altered, with irregular cracking originating from the germ end of the wheat berry. Comparison of hardness values of unimpacted control samples (8.1) and impacted samples (8.2–9.7) which showed an improvement in milling indicated that the endosperm was softer as a result of impaction at time of milling.

Experimental milling experiments to verify these findings (Figs. 3 and 4) revealed that impaction at 17% moisture produced most improvement in flour ash at higher impaction speeds (2,400 r.p.m. using a 27-in.-diameter rotor). Most improvement in ash level was noted when 20–40% of kernels were broken by impaction (determined by weighing percentage of total stock passing through a No. 10 Tyler standard sieve after impaction). This extent of breakage was achieved with lower speeds (1,600–2,000 r.p.m.) when wheat was impacted

TABLE II

EFFECT OF VARIOUS PREBREAK TREATMENTS ON EXPERIMENTAL MILLING AND BAKING BEHAVIOR OF HARD AND SOFT WINTER WHEATS

(Average and product mean values of all replicates)

Prebreak Treatment			Cracked Kernels	Flour Yield [c]	Flour Ash Extraction		Significance			Bread Score [g]
Impaction Moisture [a]	Temper Time [b]	Entol. Speed			60%	70%	1 [d]	Sig. [e]	Improvement [f]	
%	*hours*	*rpm*	%	%	%	%			%	
Ponca Seed Wheat										
16.5	24	0 [h]	...	69.8	0.40	0.43	...	...	..	88
16.5	24	0 [i]	27.1	70.1	.38	.41	1.6	x	..	96
16.5	4	2400	34.7	66.6	.37	.40	3.2	√√	9.0	90
16.5	24	2400	40.1	65.5	.36	.39	4.9	√√	6.5	95
12.0	0	1600	33.7	67.7	.37	.40	3.5	√√	4.6	91
14.0	12	0 [h]	...	70.4	.39	.43	...	...	..	93
14.0	12	1800	21.0	68.2	.37	.40	2.4	√	4.1	90
Hard Winter Wheat Mix										
16.5	24	0 [h]	...	70.1	.39	.43	...	...	..	88
16.5	24	0 [i]	23.6	67.6	.38	.42	0.0	x	..	92
16.5	4	2400	25.7	67.3	.38	.42	1.6	x	..	92
16.5	24	2400	22.6	66.1	.36	.39	5.7	√√	7.2	90
12.0	0	1600	29.7	66.5	.37	.39	1.3	x	..	89
14.0	12	0 [h]	...	69.8	.40	.43	...	...	..	..
14.0	12	1800	18.8	68.2	.37	.40	13.0	√√	8.2	82
Soft Winter Wheat										
15	24	0 [h]	...	68.1	.32	.36	...	...	..	..
15	24	0 [i]	28.3	67.3	.34	.38	1.7	x	..	..
15	4	2000	20.3	66.4	.32	.36	0.4	x	..	..
15	24	2000	27.9	64.0	.33	.37	0.4	x	..	..
11	0	1600	40.5	64.3	.32	.37	1.1	x	..	..
13	12	0 [h]	...	68.7	.34	.37	...	...	..	..
13	12	1800	34.5	64.3	0.32	0.35	0.2	x	..	..

[a] Moisture content of first temper and impaction. Following prebreak treatment, all moistures were adjusted to 16.5% (hard wheat) or 15% (soft wheat).
[b] Period between first tempering and impaction or between first and second temper (in the case of controls). The period between prebreak treatment and milling will be 24 hours less the value shown.
[c] Expressed as percent total product.
[d] Value computed from analysis of variance.
[e] √√ — Significant at 99% confidence level; √ — significant at 95% confidence level; x — insignificant at 90% confidence level.
[f] Difference below control ash at 60% extraction expressed as percent of flour ash. Improvements are indicated only in cases of statistical significance.
[g] Maximum possible bread score = 100.
[h] Control sample (not impacted).
[i] Control sample with prebreak treatment on corrugated rolls.

unwetted or at 14% moisture. Time of impaction was unimportant for samples impacted at 16.5% moisture content; less than 12 hours between initial temper and impaction were needed for improvement in ash level at moisture contents less than 14%. Other studies showed that Entoleter speeds above 2,400 r.p.m. did not improve the separation of bran from endosperm in milling, as indicated by the ash extraction values.

The four optimum impaction treatments were compared in further milling experiments to determine which treatment was most practical and desirable.

To compare roll and impaction prebreak treatments, a sample, which received a prebreak treatment on first-break rolls (speed differential 2:1), producing 20 to 30% cracked kernels, was also milled. A hard winter wheat commercial mill mix and a soft wheat were also milled in similar comparison experiments. (Speeds of 1,600, 1,800, and 2,000 r.p.m. were used for soft wheats, since this produced breakage similar to that of the treatment at the higher speeds for hard wheats.) Average values of product yield, kernel breakage, and 60–70% extraction flour ash, based on results of all milling determinations, are presented in Table II. The 60% extraction flour ash values were compared with the unimpacted control samples, using the t test for significance (17) for randomized block experiments with two treatments. The four chosen prebreak impaction treatments of hard wheats produced improvements which were significant at the 95% confidence level. Impaction at 2,400 r.p.m. immediately before milling at 16.5% moisture improved the ash value of 60% extraction flour an average of 0.028% (7% of the total flour ash at this extraction).

Impaction of soft wheat showed no significant improvement in ash extraction value at the 90% confidence level.

When the flours obtained from these various prebreak treatments on hard wheat were compared with unimpacted control flours by the baking test, all loaves were of very acceptable quality (Table II).

These studies show that prebreak impaction of tempered hard red winter wheat will improve the separation of bran from endosperm during milling. Prebreaking with rolls to the same percent breakage did not improve the ash value even at the 90% confidence level. Since the roll prebreak grinding involves considerably more shear and less compression forces than impact prebreak grinding, it is possible that it is the nature of the grindings and not the extent of kernel breakage or an additional break reduction which resulted in the better separation of bran from endosperm in subsequent milling. While the reason for this improvement is uncertain, it is possible that prebreak im-

paction cracks the wheat endosperm into agglomerates the size of coarse sizings. This cracking, induced by tempering and impaction, relieves internal endosperm stresses and thus produces a coarser break-up of the endosperm to middlings than would be obtained by reduction with break rolls alone.

When Ponca wheat is tempered to 17% moisture, it should be impacted just before milling at a rotor speed of 2,400 r.p.m., using an Entoleter with smooth stator lining (equivalent to impact velocity of 17,000 ft. per minute).

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minn. (1957).
2. Anderson, J. E. Some tests with the Entoleter. Assoc. Operative Millers, Tech. Bull. No. 2, pp. 393–400. Sosland Press, Inc.: Kansas City, Mo. (1943).
3. Borth, K. Bas Schälproblem in der Getreidemullerei. Die Mühle **80** (10): 130–131 (1943).
4. Eisgruber, G. Die Nasschälung-Getreideanthäutung. Mühlen-Zeitung **3** (11): 216 (1949).
5. Eschaus, K. H. Weizenschälen oder-bürsten. Die Müllerei **6** (9): 121–122 (1953).
6. Flechsig, J. Die Berechnung der Nehlausbeute aus den Ergebnissen des Mahlversuches und aus analytischen Daten. Getreide u. Mehl **4** (8): 60–62 (1954).
7. Geddes, W. F., and West, H. E. A statistical study of the reliability of the experimental milling test. Sci. Agr. **10:** 333–343 (1930).
8. Geddes, W. F., and Aitken, T. R. The comparative quality of flours from corresponding wheats milled on an Allis-Chalmers experimental mill and a Brabender automatic laboratory mill. Cereal Chem. **14:** 511–524 (1937).
9. Grosh, G. M., and Milner, M. Water penetration and internal cracking in tempered wheat grains. Cereal Chem. **36:** 260–273 (1959).
10. Hibbs, A. N., Shellenberger, J. A., and Pence, R. O. The theory of impact grinding with centrifugal machines. Trans. Am. Assoc. Cereal Chemists **6:** 26–31 (1948).
11. Hinton, J. J. C. The distribution of ash in the wheat kernel. Cereal Chem. **36:** 19–31 (1959).
12. Hopf, L. Die halbfeuchte Schälung. Die Müllerei **3:** 861–863 (1950).
13. Melges, B. W. Schälmaschine mit Schälrotoren. Die Mühle **90:** 367 (1953).
14. Schulz, A. Beeinflussung des keimgehalfes von Weizen durch Wäsche und Schälung. Getreide u. Mehl **2:** 62–64 (1952).
15. Seeborg, E. F. Evaluation of wheat milling characteristics by laboratory methods. Trans. Am. Assoc. Cereal Chemists **11** (1): 1–5 (1953).
16. Smeets, H. S., and Cleve, H. Determination of conditioning by measuring softness. Milling Production **21** (4): 5, 12, 13, 16 (April 1956).
17. Snedecor, G. W. Statistical methods (4th ed.), pp. 263–265. Iowa State College Press: Ames, Iowa (1946).
18. Uhlmann, W. Ein neues Schälverfahren. Die Mühle **90:** 144 (1953).
19. Ziegler, E. The effect on diastatic activity of surface pressure, differential, and temperature of the reduction rolls in milling. Cereal Chem. **17:** 668–679 (1940).

THE "REMIX" BAKING TEST[1]

G. N. IRVINE AND MARION E. MCMULLAN

ABSTRACT

A "Remix" baking test, designed to measure strength, is described. A malt-phosphate-bromate formula is used, and the dough is mixed for 3.5 minutes in the GRL mixer or 2 minutes in the Swanson mixer. After fermenting for 165 minutes without punching, the dough is remixed for 2.5 minutes in the GRL or 80 seconds in the Swanson mixer. A recovery time of 25 minutes is allowed, and the dough is then sheeted, molded, panned, proofed for 55 minutes, and baked.

The new method requires no skilled punching, facilitates mechanized molding, is reproducible, and is relatively insensitive to minor variations in timing. The Remix method detects extreme strength more readily, and discriminates more widely between strong and weak flours, than the regular malt-phosphate-bromate straight dough method. Typical results comparing the Remix method with other methods of assessing strength are presented.

The baking test described in this paper was developed specifically to provide a measurement of flour strength in terms of loaf volume at satisfactory levels of loaf appearance and crumb characteristics. Interest shown in this test by others, since publication of a brief note (3), suggests that it may prove a useful addition to test baking methods now in use in various countries.

The meaning of "strength," as applied to wheat or flour, is elusive; the concept is difficult to define in general terms and more especially in terms of measurable properties. Throughout some countries, and in certain parts of other countries, the indigenous wheat is incapable of producing a loaf of the quality demanded by local consumers. A stronger wheat must then be used to blend with the indigenous wheat, and this practice gives rise to a general classification of wheats in terms of strength; the strongest wheat may be defined as the one required in smallest amount to yield a loaf of a given standard quality when blended with the indigenous wheat. We believe that this general definition would be widely accepted in many countries.

Although the hard wheats of the world vary widely in strength, depending on the variety and the environment in which they are grown, the strongest types generally have a number of qualities in common in addition to their ability to support weaker wheats. They tend: to be of high protein content; to have a high water absorption which persists throughout baking; to give doughs that are springy,

[1] Manuscript received November 13, 1959. Paper No. 184 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba. Presented in part at the 44th annual meeting, Washington, D.C., May 1959.

elastic, and extensible; and to show wide tolerance to conditions of mixing, of fermentation, and of dough handling. The concept of strength includes these associated characteristics in varying degree, depending upon what is required of the strong wheat in its supporting role. More detailed definitions of strength, in terms of measurements of these properties, can thus be developed, but tend to differ in emphasis from country to country.

In this laboratory, measurements of strength are required for assessing new varieties, the quality of each new crop, and the qualities of the individual grades currently being exported. While a wide range of analytical and dough-testing methods have been used, emphasis has traditionally been placed in the results of baking tests.

The test baking method which has long been in use in this laboratory is a fixed-formula, fixed-mixing-time, straight-dough method following the AACC straight-dough procedure (2) using 100 g. of flour; the ingredients are the same as those listed for the Remix method in a later section, except that the bromate level is 1.0 mg. Although it has probably been recognized as one of the best of the baking tests for strong wheats (1,7,8), this method has not proved entirely satisfactory. Medium-strength wheats of high protein content often give better results than strong wheats of equally high protein content. It thus appears that the method is not sufficiently rigorous for proper development of strong wheats. However, increasing the mixing time or speed yields bucky doughs that do not rise sufficiently during proofing and baking to produce loaves of good volume. Moreover, if the time schedule and conditions for punching, fermentation, molding, and proofing are fixed, the experimental baker avoids buckiness by reducing mixing time; the method then becomes even less satisfactory for the strongest wheats.

Some two years ago, a new baking test, the "Remix Method," was developed in this laboratory and briefly described in the Annual Report for 1957 (3). The Remix method is a rigorous fixed-procedure method that, with strong flours, gives loaves of excellent volume, appearance, crumb grain, texture, and color; weakness in the flour is reflected first in lower volume and ultimately in poorer dough-handling qualities and poorer crumb characteristics as well. The method is designed primarily to provide better differentiation among strong and medium-strong flours. Specifications for the method, comments on its main characteristics, and some comparisons of the Remix method with the baking method formerly used (hereinafter referred to as the MPB method) and with other methods of assessing strength, are reported in this paper.

Method

Equipment: (1) Mixer. The GRL mixer (9) run at 130 r.p.m. is preferred, but the Swanson mixer is also satisfactory. (All other equipment as for the AACC Straight-Dough Method (2).)

Formula:

Flour (14% moisture basis)	100.0 g.
Water, distilled	variable
Yeast, compressed	3.0 g.
Sugar	2.5 g.
Salt	1.0 g.
Malt syrup (250° Lintner)	0.3 g.
Ammonium dihydrogen phosphate	0.1 g.
Potassium bromate	1.5 mg.

Procedure: (1) Mix ingredients for 3.5 minutes in GRL mixer or 2.0 minutes in Swanson mixer.
(2) Ferment for 165 minutes at 30°C.
(3) Remix for 2.5 minutes in GRL or 1 minute 20 seconds in Swanson mixer.
(4) Ferment for 25 minutes (recovery time).
(5) Sheet and mold.
(6) Proof for 55 minutes at 30°C.
(7) Bake 25 minutes at 220°C.

Comments. The formula for the Remix method is identical with that for the MPB method, except that the bromate level has been increased from 1.0 to 1.5 mg. Total time for the test is increased by 10 minutes. Initial mixing time and speed were increased to obtain proper development with strong flours, and the resulting buckiness is removed by remixing after fermentation. Hand punching is eliminated, an important advantage since considerable skill and experience are required to obtain reproducible results. Moreover, the remixed doughs, even from strong wheats, are softer and more extensible, and are thus better suited for mechanical molding.[2] By eliminating hand punching and hand molding, the human factor in test baking can be substantially reduced if not entirely eliminated.

TABLE I

VARIATION OF LOAF VOLUME WITH BROMATE LEVEL AND REMIXING TIME

REMIXING TIME	BROMATE LEVEL (mg.)			
	0	1.0	2.0	3.0
minutes	*cc*	*cc*	*cc*	*cc*
1.5	675	755	795	760
2.5	665	770	760	715
3.5	730	740	690	690

Experiments made while the method was being developed led to the following conclusions. With strong flours the method is not sensi-

[2] All results reported in this paper were obtained with hand molding. Subsequently a modified version of a standard laboratory molder was constructed and is now in routine use with this method.

tive to small variations in the amount of initial mixing, nor is there any appreciable interaction between initial mixing and bromate level. As would be expected from the well-known studies of Freilich and Frey (6), there is an interaction between bromate level and remixing; this behavior is shown in Table I. As the bromate level is increased up to about 3.0 mg., the remixing time required to produce optimum loaf properties is decreased. A good loaf is produced with no bromate by longer remixing, but the volume is not as large as that obtained with some bromate and shorter remixing. As the bromate level is increased at a given remixing time, or as remixing time is increased at a given bromate level, doughs become more slack and sticky. These considerations led to adoption of 2.5 minutes' remixing and 1.5 mg. bromate as practical working levels.

The method is not sensitive to excess bromate; increases in bromate beyond the optimum do not severely affect loaf properties. This is in marked contrast to the MPB method which is quite sensitive to bromate level, particularly with high-protein flours (1). The beneficial effects of remixing overbromated or bucky doughs have, of course, been known for many years (5,6).

There is a differential response to fermentation time (time between mixing and remixing) with differences in the bromate level, as shown in Table II; this phenomenon is common to most baking methods (1,4). With no bromate, loaf volume increased as fermenta-

TABLE II

VARIATION OF LOAF VOLUME WITH BROMATE LEVEL AND FERMENTATION TIME

Fermentation Time	Bromate Level (mg.)		
	0	1.0	2.0
hours	*cc*	*cc*	*cc*
2.25	715	850	920
2.75	740	885	915
3.25	755	865	915

tion time increased. With 1.0 mg. bromate, optimum loaves were obtained at 2.75 hours; and at 2.0 mg., loaf volume did not vary over the range of fermentation times. A compromise of 2.75 hours with 1.5 mg. of bromate was selected.

Immediately after remixing, doughs tend to be slack, sticky, and difficult to handle, and this tendency is greater with weaker flours. After a 25-minute recovery time, even weak doughs can be readily handled, and there appeared to be no advantage in shortening this time for medium and strong doughs.

With strong flours and no bromate, loaf volume, texture, and

color improve with increasing mixing time, fermentation time, and remixing time. But for any bromate level between 1.5 and 3.0 mg., loaf characteristics change little over reasonable ranges of mixing, fermentation, and remixing times. Moreover, as pointed out earlier, the method also tolerates excess bromate. For these reasons it appears that the method is reproducible without close standardization; minor variations in procedure and formula do not tend to disturb the rank order in which a series of samples are placed by the Remix method.

Comparisons of the Remix and Other Tests

The differential response of flours of various baking strengths to the Remix method, the MPB method, and certain other tests is illustrated in the following subsections. Comparisons of the two methods were made with: 1) 14 wheat varieties; 2) two sets of samples representing the principal grades of Canadian spring wheat; 3) five types of commercial winter wheat; 4) four commercially milled domestic flours.

All wheats were milled on an experimental Allis-Chalmers mill to yield a standard feed flour based on an extraction of 73% for top-grade Canadian wheat. Loaf volume, Zeleny tests, and extensigraph areas were determined in duplicate, but farinograph data represent single tests.

Varieties. Composite samples of each of 14 varieties, grown at several stations in Western Canada, were studied: 1) Rescue × (Renown × S615); 2) Ceres; 3) Saunders × CT 219; 4) Rescue; 5) Rescue × Cadet; 6) (Renown × S615) × Rescue; 7) Cadet; 8) Timstein; 9) S615; 10) Mida × (McMurachy × Exchange); 11) Egypt NA95; 12) Exchange; 13) 1088 × Yaqui; 14) Kenya 321 BT1B1. These varieties were selected, from a larger series in use in the Canadian plant breeding program, as typical of various degrees of baking strength. Protein contents of flour, loaf volumes by the two baking methods, and the results of Zeleny, farinograph, extensigraph, and absorption tests, are given in Table III.

The varieties are listed in order of decreasing Remix loaf volume and are divided into four groups tentatively classed as very strong, strong, medium, and weak. The better-known varieties Thatcher, Selkirk, and Marquis fit into intermediate places in this classification, with Thatcher between the strong and very strong and Selkirk and Marquis between the strong and medium groups. Though there are discrepancies within each group, the mean data for groups show that the Zeleny test, farinograph development times and weakening, and

TABLE III

BAKING AND SUPPLEMENTARY DATA ON VARIETY SERIES

Group No.	Variety No.	Flour Protein	Loaf Volume		Zeleny Test	Farinograph		Extensigram Area	Absorption		
			Remix	MPB		Dev. Time	Weakening		Farinograph	Remix	MPB
		%	*cc*	*cc*		*minutes*	*B.u.*	cm^2	%	%	%
1. Very strong	1	14.0	985	730	68	7.2	34	198	64	58	60
	2	15.4	975	760	62	6.8	47	144	72	63	66
	3	13.5	965	665	68	8.0	14	200	65	61	61
	4	14.7	950	760	69	6.0	37	203	63	56	59
	Mean	14.4	969	729	67	7.0	33	186	66	60	62
2. Strong	5	15.3	915	930	61	6.2	33	170	67	61	63
	6	15.0	915	955	71	6.8	31	206	65	59	61
	7	14.8	865	910	69	6.8	42	134	72	65	66
	Mean	15.0	898	932	64	6.6	35	170	68	62	63
3. Medium	8	14.6	635	920	51	5.0	81	102	69	54	65
	9	15.0	555	915	42	3.2	107	55	67	53	63
	10	14.6	525	840	43	3.2	100	60	67	51	63
	11	14.7	510	830	38	3.8	80	29	70	53	62
	Mean	14.7	556	876	44	3.8	92	62	68	53	63
4. Weak	12	12.6	490	630	26	2.0	165	26	62	49	58
	13	14.0	470	660	23	2.2	150	..	64	48	55
	14	12.5	455	665	39	2.8	131	46	61	49	57
	Mean	13.0	472	652	29	2.3	149	36	62	49	57

extensigraph area all agree in placing the four groups in the same order as does the Remix loaf volume. Moreover, for the first three groups, which differ little in mean protein content, all these tests obviously reflect some strength factor (or factors) other than protein content. Indeed, this conclusion also applies to the fourth group, since the mean loaf volume by the Remix method is lower than would be expected solely from consideration of the lower mean protein level.

In comparison with all other tests, the MPB loaf volumes establish a different rank order for the four groups. The strong group is placed first, followed closely by the medium group, with the very strong group substantially lower in third position, and a further substantial decrease for the weak group in fourth position. Thus it seems reasonable to infer that the MPB method favors the strong and medium-strong groups, penalizes the very strong group, and does justice to the weak group. The differential response of samples to the two baking methods is worth emphasizing. For the very strong wheats, Remix volumes are larger than MPB volumes; for the strong types, both methods give similar volumes; for the intermediate-range and weak wheats, however, Remix volumes are smaller than MPB volumes. This pattern is also consistent in the series that follow.

The absorption data are also interesting. Baking absorption, as measured by the feel of the dough at panning, is much the same for the first three groups with the MPB method, though lower for the fourth group. This order also applies for farinograph absorption. For the Remix method, baking absorption falls off more rapidly for the third group and especially for the fourth group; these flours would be unmanageable after remixing at higher absorptions.

Spring Wheat Grade Samples. Studies were made of two sets of samples representing grades of Canadian Hard Red Spring wheat. The first set comprised composites of grades 1 to 4 Manitoba Northern and No. 5 wheat collected at Vancouver in 1958; the second set comprised composites of wheat of the 1959 crop for grades 1 to 4 Manitoba Northern, and No. 4 Special. The two sets differ by about 1.5 percentage units in average protein content.

Data for the first set are given in the upper half of Table IV. The Remix loaf volumes rank the samples in order of grade, although some of the differences between grades are too small to be significant. The MPB method responds best to grade 3 and does not reflect the true difference in strength between grade 1 and No. 5. In this set, extensigram areas are not related to grade. Absorption data are included mainly to show that these were the same for the two methods for each of the top three grades, but differed by 1% for the last two.

TABLE IV
BAKING AND SUPPLEMENTARY DATA ON CANADIAN SPRING WHEATS

Grade	Flour Protein	Loaf Volume		Farinograph		Extensigraph Area	Absorption	
		Remix	MPB	Dev. Time	Weakening		Remix	MPB
	%	cc	cc	minutes	B.u.	cm^2	%	%
No. 1 Northern	12.0	765	710	6.0	45	123	61	61
No. 2 Northern	12.1	745	710	6.0	45	111	60	60
No. 3 Northern	12.2	740	760	5.5	55	119	60	60
No. 4 Northern	12.1	690	710	5.5	60	111	61	62
No. 5 Wheat	11.7	680	680	5.5	70	117	60	61
No. 4 Special	15.9	980	900	7.5	20	161	63	63
No. 1 Northern	13.3	850	765	6.0	45	134	60	60
No. 2 Northern	13.6	865	830	6.0	35	131	61	61
No. 3 Northern	13.5	775	815	6.0	50	123	61	61
No. 4 Northern	13.0	725	760	6.0	55	96	59	59

The lower half of Table IV shows data for a set of grade composites representing higher protein levels. Number 4 Special, which has the highest protein content, is listed first; this grade contains mature wheat degraded because of low bushel weight (53 to 55 lb.) caused, in this case, by drought.

With an advantage of over 2% in protein, No. 4 Special is sufficiently strong to be placed first by all methods including MPB loaf volumes. For the remaining grades, Remix volumes follow grade quite well, except for a slight perturbation due to protein content. The Remix method, farinograph weakening, and extensigram area agree. The MPB method favors the lower grades at the expense of No. 1 Northern and shows similar volumes for the highest and lowest grades. The spread in loaf volumes between No. 4 Special and No. 4 Northern for the Remix method is 255 cc., whereas with the MPB method it is only 140 cc. Thus, this set of data indicate the superior ability of the Remix method to discriminate between samples of similar character but of slightly different strength.

Winter Wheats. Data for five types of winter wheat, from three different countries, are given in Table V. The Remix loaf volumes and the farinograph data place these samples in the same order; extensigram areas also agree, save for reversing of Argentine Southern and U.S. Dark Hard Winter. These wheats were composites made up from samples of seven to ten individual cargoes of each type unloaded in Europe in late 1957 or in 1958 and are reasonably representative of these classes of wheat as imported into Europe during this period. They comprise wheat types whose general order of strength is well known to European millers; we believe that these millers would agree to the rank order of strength established by the Remix method. Dur-

TABLE V

BAKING AND SUPPLEMENTARY DATA ON WINTER WHEAT CARGO COMPOSITES

TYPE	FLOUR PROTEIN	LOAF VOLUME		FARINOGRAPH		EXTENSIGRAPH AREA	ABSORPTION	
		Remix	MPB	Dev. Time	Weakening		Remix	MPB
	%	cc	cc	minutes	B.u.	cm^2	%	%
Argentine Upriver	11.6	760	670	7.0	35	162	58	58
Argentine Southern	10.8	720	635	6.5	50	122	59	59
U.S. Dark Hard Winter	13.3	700	770	5.5	80	130	57	59
U.S. Hard Winter	11.6	665	715	5.5	85	117	55	57
Russian	11.5	625	660	4.5	95	74	51	57

ing the period specified, Argentine Upriver was generally classed as the strongest winter wheat imported into the European market, followed by Argentine Southern and U.S. Hard Winter of high protein content. Imports of Russian wheat were generally of lower baking strength than imports of U.S. Hard Winter of average protein content. This classification is in substantial agreement with data published by Shellenberger (11) in his study of wheats imported into Europe during 1955 and the early part of 1956.

In contrast to the other methods, the MPB loaf volumes fail to reflect the strength of the two Argentine types. Indeed, for this series of samples, MPB volumes appear to be influenced mainly by protein contents.

Commercial Flours. The series of commercial flours comprised three baker's patent flours of 13.4, 12.6, and 12.2% protein, and a straight-grade flour of 12.0% protein milled from No. 5 wheat. The flours were treated at the mill and are more fully matured than the laboratory-milled flours considered in previous subsections; the extensigraph curves indicated that the low-protein patent flour had been given the most oxidation treatment.

Data in Table VI show that both baking methods rank the patent flours in order of their protein content, but that the MPB method ranks the weaker No. 5 flour above all but the high-protein patent, which showed the mildest oxidation treatment. The Remix method, on the other hand, ranks the No. 5 flour considerably below any of the patent flours; the spread between the strongest and weakest flours is 180 cc. by the Remix method but only 25 cc. by the MPB method.

For the patent flours, Remix volumes are higher than MPB volumes. The differences between the two methods are related to the ap-

TABLE VI

BAKING AND SUPPLEMENTARY DATA ON COMMERCIAL FLOURS

Flour	Protein	Loaf Volume		Farinograph		Extensigraph Area
		Remix	MPB	Dev. Time	Weakening	
	%	cc	cc	*minutes*	*B.u.*	cm^2
High-protein Patent	13.4	810	740	7.0	45	172
Medium-protein Patent	12.6	765	675	7.0	40	185
Low-protein Patent	12.2	745	615	6.5	45	202
Straight-grade No. 5	12.0	630	715	3.5	90	80

parent degree of oxidation: the low-protein patent shows a difference of 130 cc. in volume, has the greatest extensigram area, the greatest resistance, and the least extensibility; the high-protein patent shows the smallest difference, 70 cc., and has the smallest extensigram area, together with the lowest resistance and greatest extensibility.

These data tend to confirm the belief that the Remix method is relatively insensitive to the degree of oxidation of the flour but is highly sensitive to the type of gluten quality that seems to be associated with flour strength. Thus, differences in gluten quality do not tend to be masked by other factors.

Discussion

In general, test baking methods are of two types: those employing fixed processing conditions and fixed formulas and those in which processing and/or formula may be varied to suit the needs of the flour being tested. In the fixed type of baking test, a rigid standard is, in effect, being set. All flours, to give acceptable results, must be equal to or better than this standard; any flour which differs significantly in type from the standard is penalized although it might be equally useful, for many purposes, to those types which meet the standard. Two very important aspects of such a test are the level of strength at which the fixed procedure gives optimum results, and the latitude or tolerance of the test to variables other than inherent strength.

Both the Remix and the MPB methods are of this fixed type. From the results reported here (and from a great deal of other experience with both methods), it appears that the Remix method as outlined here is "tuned" to a higher level of strength than is the MPB method; it also appears to have a greater degree of tolerance to variables other than inherent strength, so that the results, as measured by loaf volume, reflect this factor principally, and interacting factors such as protein content and degree of oxidation do not tend to interfere seriously with the measurement of this principal objective.

The Remix method has proved to be most useful in the assessment of strength in the areas of testing for which examples are cited in this paper. In some of these areas, notably the variety testing, which involves a very wide range of gluten quality, there are close correlations between Remix loaf volumes and the results of the farinograph, extensigraph, and Zeleny tests. In other series, however, where the general level of strength is more uniform, these latter tests appear to give less useful specific information than the Remix test.

Introduction of remixing gives the test method something in common with the sponge and dough method so widely used in commercial bakeries. It is also interesting that commercial equipment and procedure almost identical with the Remix method has recently been described (10). Moreover, continuous doughmaking processes, insofar as they involve recovery after dough development, are also partially simulated by the Remix method. It may therefore have applications in testing the particular strength requirements of flour for highly mechanized continuous processes. There are other testing areas and other testing objectives which are doubtless better served by variable-procedure baking methods.

Literature Cited

1. Aitken, T. R., and Geddes, W. F. The behavior of strong flours of widely varying protein content when subjected to normal and severe baking procedures. Cereal Chem. 11: 487–504 (1934).
2. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
3. Anderson, J. A. Remixed straight dough baking test. Grain Research Laboratory, 1957 Report, p. 7.
4. Doose, O., and Wolter, K. The influence of flour treated with potassium bromate and ammonium persulfate upon dough produced by various methods. Trans. Am. Assoc. Cereal Chemists 13: 130–147 (1955).
5. Frank, E. N. Some effects of reworking fermenting doughs. Cereal Chem. 19: 379–383 (1942).
6. Freilich, J., and Frey, C. N. Dough oxidation and mixing studies. II. Effects of remixing after fermentation. Cereal Chem. 16: 495–502 (1939).
7. Geddes, W. F., Aitken, T. R., and Fisher, M. H. The relation between the normal farinogram and the baking strength of Western Canadian wheat. Cereal Chem. 17: 528–551 (1940).
8. Geddes, W. F., and McCalla, A. G. Comparison of a bromate and malt-phosphate-bromate formula in testing wheat quality for the plant breeder. Cereal Chem. 11: 384–395 (1934).
9. Hlynka, I., and Anderson, J. A. Laboratory dough mixer with an air-tight bowl. Cereal Chem. 32: 83–87 (1955).
10. Marnett, L. F. The ful-flavor process . . . theory and practice. Baker's Digest 33 (4), 54–58 (1959).
11. Shellenberger, J. A. Survey of the quality of European wheat imports. Agr. Exp. Sta., Kansas State College, Bull. 396 (1958).

SOME CHEMICAL CHANGES WHICH ACCOMPANY THE BROWNING OF CANNED BREAD DURING STORAGE[1]

RUSSELL J. STENBERG[2] AND W. F. GEDDES

ABSTRACT

The rate of browning of canned bread, as determined at 600 mμ with a Beckman Model DU spectrophotometer equipped with a reflectance attachment, increased with the temperature of storage. At 25°C. there was only very slight browning after 120 days, but at 35°C. appreciable browning had occurred at 100 days' storage. At 50° and 75°C. browning was progressively much more rapid. No appreciable biochemical changes were noted in bread stored at −15° and 25°C. At 35°C., however, the pH decreased, whereas the total soluble nitrogen, titratable acidity, and amino nitrogen increased in the samples stored for 100 days. These biochemical changes were much more marked at 50° and 75°C. Lysine nitrogen decreased with an increase in storage time at 50° and 75°C. Total reducing substances remained essentially constant. Bacteriological tests indicated that these chemical changes were not likely to be due to bacterial action. The brown pigment could not be extracted with water or with a number of organic solvents. Acetylation gave a brown reaction mixture with both white and brown crumb; water then extracted the brown pigments from both samples, yielding solutions of similar absorbance and amino nitrogen content.

The development of a satisfactory method of canning white bread by the Quartermaster Corps in 1946 provided a means of supplying bread in field rations[3]. Although canned bread remained palatable for several months, it eventually developed a yellowish-brown color and acquired a slightly unpleasant odor and taste. These phenomena are likely caused by nonenzymic browning reactions which commonly occur upon the storage of heat-processed foods.

Several theories have been proposed for the mechanism of the chemical reactions which lead to the development of nonenzymatic browning in heat-processed foods. As early as 1912, Maillard (16) observed that upon heating, a mixture of amino acids and reducing sugars became dark in color. Hodge (6) has recently reviewed various lines of evidence concerning the details of the reaction. Lea and

[1] Manuscript received January 13, 1960. Contribution from the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota. Paper No. 4209 of the Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper represent part of a thesis presented to the Graduate School of the University of Minnesota by Russell J. Stenberg in partial fulfillment of the requirements for the Ph.D. degree, May 1950.

This paper reports research undertaken in cooperation with the Quartermaster Food & Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned No. 1078 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

[2] Present address: Northern Utilization Research and Development Division, Agricultural Research Service, USDA, Peoria, Ill.

[3] Holton, Paul; from a lecture before Midwest Section, AACC, and given in AACC News Letter No. 67, May 1946, p. 81.

Hannan (11,12,13), employing casein and glucose as model reactants, observed a decrease in the ε-amino groups of lysine and the formation of bound glucose; later, the complex darkened in color, and lysine could not be recovered upon acid hydrolysis of the reaction product (10).

Protein-sugar reactions of the Maillard type are frequently involved in the browning of processed foods. Among other factors, it is generally accepted that increases in active acidity retard the rate of discoloration of some food products (2,9,19). The researches of Haas and Stadtman (5) and of Lewis *et al.* (14) led to the postulation that organic acids may be involved in the browning of dehydrated fruits.

To serve as a basis for a later investigation of the effect of formula variations on the browning of canned bread, the preliminary exploratory studies reported in this paper were undertaken. The study is divided into two sections: 1) gross changes in the chemical composition of canned white bread as browning occurred during storage at different temperatures, and 2) attempts to extract the brown pigments from the bread after storage.

Materials and Methods

Preparation and Storage of Canned Bread. The canned bread was prepared according to the following formula[4], supplied by the Quartermaster Food & Container Institute.

Ingredients[a]	*Parts by Weight*
Flour	100.00
Salt	1.75
Sucrose	4.00
Shortening	4.00
Nonfat dry milk	2.00
Monocalcium phosphate	0.25
Calcium propionate	0.125
Yeast, compressed	2.00
Water	60.00

[a] The flour, a low-protein short patent, milled from hard red winter wheat, and hydrogenated vegetable shortening (100-hour stability) were supplied by the Institute.

The bread was made by a 100% sponge procedure; 2,835 g. of flour and the proportional quantities of salt, monocalcium phosphate, yeast, and water were placed in an 8-qt. round-bottomed bowl and mixed for 2.5 minutes at slow speed in a Model C-10 Hobart mixer

[4] Subsequent to this work, the Institute developed a new formula for canned bread (Chicago Quartermaster Depot, Military Specification; Bread, Canned, MIL-B-1070B; 1953) which was designed to yield a product having a lower pH and moisture content than that produced by the formula used in this study.

equipped with two hooks. The sponge was fermented 135 minutes at 30°C.; the remaining ingredients were added and mixed for 1 minute at low speed and 3 minutes at second speed. The remixed dough was immediately scaled into 15 pieces each weighing 326 g., which were then rolled in hydrogenated shortening and placed in No. 2½ lacquered cans. The cans were clinched and proofed (about 44 minutes) at 30°C. until the dough was 1.5 in. from the top of the cans. The dough was baked at 232°C. for 30 minutes. The bread was allowed to cool for 5 minutes after which the cans were hermetically sealed and immersed in running tapwater for 45 minutes.

Storage and Preparation of Bread Crumb. The canned bread was stored at temperatures of −15°, 25°, 35°, 50°, and 75°C. After various periods of storage the cans were opened, the crust was removed from the bread, and the crumb cut into thin slices and dried at room temperature to less than 10% moisture. The crumb was then ground in a semimicro Wiley mill to pass a No. 40 sieve.

Changes in the Chemical Composition of Canned Bread with Browning

The extent of browning of the dried crumb was measured and analyses were made for total soluble nitrogen, free amino nitrogen, titratable acidity, ε-amino groups of lysine, reducing substances, and pH.

Methods. A measure of the browning of the bread crumb was obtained by means of a Beckman Model DU spectrophotometer with a reflectance attachment. A block of magnesium carbonate with a freshly scraped surface was used as a standard (reflectance = 1.000). The samples were prepared by packing the finely ground crumb into dishes 1.5 in. square and 0.5 in. deep and carefully smoothing the surface with a spatula. Reflectance was measured at 600 mμ[5]. Good reproducibility of surfaces was obtained, the deviation in the readings taken from the same lot of bread being less than 1%.

[5] Reflectance measurements were first made on a control sample of dried bread crumb and on a heavily browned sample (stored at 75°C. for 35 days) at a series of wave lengths between 350 and 700 mμ. Representative values were:

	Reflectance	
Wave length	*Control crumb*	*Browned crumb*
mμ		
350	0.303	0.035
500	0.684	0.094
600	0.766	0.237
700	0.814	0.446

Since the accuracy of any photoelectric measurement falls off rapidly when the transmission of light is less than 15%, a wave length of 600 mμ was selected. After the completion of this work Larson *et al.* (8) have utilized the Hunter Color and Color-Difference Meter for following the browning of bread crumb, a method which probably gave more accurate results.

Total soluble nitrogen was determined by extracting 4.0 g. of dried crumb in a 125-ml. Erlenmeyer flask with 20 ml. of water for 60 minutes at room temperature with intermittent shaking. After centrifugation, the supernatant was filtered and analyzed for nitrogen by a micro-Kjeldahl procedure. Digestion was carried out with selenium oxychloride in sulfuric acid (perchloric acid was omitted) according to the method of Pepkowitz and Shive (18). Distillation of the digest and titration were conducted by the procedure of Ma and Zuazaga (15).

The titratable acidity and free amino nitrogen of the dried crumb were determined on a 20-g. sample by the modified Sorenson formol titration method given in *Cereal Laboratory Methods* (1).

Basic lysine nitrogen of the crumb was determined by a microbiological assay procedure[6] described by Zittle and Eldred (22).

Reducing substances were determined in a 2.5-g. sample by the alkaline ferricyanide procedure for reducing substances in flour (1). Since numerous reducing substances, in addition to maltose, were probably present in the bread crumb, the results are reported in terms of the quantity of ferricyanide reduced.

The pH of the crumb was measured by the method for bread (1), employing a Coleman Model 3 pH electrometer.

To ascertain the possible role of bacteria as a cause of the chemical changes occurring in canned bread, plate counts were made on bread crumb from two cans of bread which had been stored 2 years at 25°C. using dextrose-tryptone agar[7] which is recommended by the National Canners' Association for the determination of flat-sour organisms in flour, starch, and sugar. Inoculations were made with successive dilutions of the bread crumb (as removed from the cans) in sterile water. A fluid thioglycollate medium which is capable of supporting many types of aerobic and anaerobic organisms was also inoculated with successive dilutions of the bread-crumb dispersion.[8]

Results

The results of the reflectance and biochemical measurements on the bread crumb are recorded in Table I. The reflectance data were in accord with the browning of the crumb, as judged visually. At 25°C. there was only very slight browning after 120 days of storage, but at 35°C. appreciable browning had occurred at 100 days of storage.

[6] The authors are indebted to the General Mills Research Laboratories, Minneapolis, Minn., for carrying out these analyses.

[7] Difco Laboratories, Inc.: "Manual of Dehydrated Culture Media and Reagents" (7th ed.). Detroit, Mich. (1944).

[8] Dr. J. J. Jezeski, Department of Dairy Husbandry, University of Minnesota, kindly performed these studies.

With storage temperatures of 50° and 75°C., browning was progressively much more rapid; at the latter temperature, browning was so extensive that the measurements were discontinued after 35 days of storage.

No appreciable biochemical changes were noted in the bread crumb stored at −15° and 25°C. At 35°C., however, the pH decreased, whereas the total soluble nitrogen, titratable acidity, and amino nitrogen increased in the samples stored for 100 days, a time which corresponded with the development of a brown color. At storage temperatures of 50° and 75°C. these biochemical changes were much more marked, particularly at 75°C. The rate of increase in total soluble nitrogen was the same as the rate of increase in amino nitrogen at the three highest storage temperatures, revealing a break-

TABLE I

EFFECT OF TIME AND TEMPERATURE OF STORAGE OF CANNED WHITE BREAD ON THE BROWNING AND THE BIOCHEMICAL PROPERTIES OF THE CRUMB OF THE BREAD[a]

TEMPERATURE	DAYS	pH OF CRUMB EXTRACT	TOTAL SOLUBLE NITROGEN	TITRATABLE ACIDITY[b]	AMINO NITROGEN	REDUCING SUBSTANCES[c]	REFLECTANCE[d]	LYSINE NITROGEN
°C			mg		mg			mg
..	0	5.7	9.1	0.50	0.13	28	....	....
−15	14	5.7	8.9	0.50	.16	27	....	....
−15	48	5.7	9.6	0.51	.15	27	....	....
−15	100	5.6	9.1	0.51	.17	28	....	....
−15	200	5.6	8.6	0.57	.18	28	....	....
..	0	5.7	9.4	0.50	.16	29	0.760	2.43
25	11	5.7	8.5	0.51	.15	29	.765	....
25	31	5.7	8.4	0.50	.16	29	.767	....
25	60	5.7	8.3	0.49	.17	30	.768	....
25	120	5.5	9.2	0.55	.17	30	.755	2.51
25	200	5.5	9.4	0.58	.18	30	.750	....
..	0	5.8	9.3	0.46	.17	29	.763	2.45
35	10	5.7	9.1	0.50	.20	29	.752	....
35	27	5.6	8.8	0.53	.15	29	.774	....
35	50	5.7	8.6	0.48	.16	28	.771	2.64
35	100	5.4	13.2	0.66	.24	28	.738	....
35	150	5.2	14.1	0.81	.26	28	.720	2.84
..	0	5.7	8.7	0.35	.13	27	.762	2.73
50	0	5.7	8.8	0.38	.14	27	.744	....
50	11	5.6	8.9	0.39	.15	28	.706	....
50	20	5.5	9.8	0.43	.21	27	.666	2.45
50	40	5.4	11.0	0.47	.21	26	.665	....
50	60	5.4	13.0	0.51	.18	27	.635	2.21
..	0	5.8	8.9	0.35	.17	28	.766	2.81
75	5	5.4	14.5	0.49	.24	27	.601	....
75	10	5.2	21.0	0.67	.34	27	.526	....
75	17	5.0	28.6	0.80	.44	26	.417	1.61
75	25	4.9	37.6	0.93	.67	26	.328	....
75	35	4.8	45.5	1.12	0.85	24	0.237	1.03

[a] All data are expressed on the basis of 1 g. dry crumb.
[b] Expressed as ml. 0.14*N* base neutralized.
[c] Expressed as ml. 0.1*N* ferricyanide reduced.
[d] Measured at 600 mμ.

down of the protein. Lysine nitrogen decreased with an increase in the storage time at 50° and 75°C., particularly at the latter temperature.

Total reducing substances remained essentially constant except, perhaps, for a very slight decrease after 35 days of storage at 75°C.

Limited bacteriological tests indicated that the chemical changes which occurred during storage could not be ascribed to bacterial action. A can of bread stored 2 years at room temperature which had a distinctly yellow crumb and a cheeselike odor gave negative tests with both the dextrose-tryptone agar and the fluid thioglycollate medium. Another can stored under similar conditions had a bacterial count of only about 16,000 per g.

Attempted Extraction of the Brown Pigments of Dried Bread Crumb

Investigators have succeeded in extracting the brown pigments from such food products as nonfat dry milk, dried whey, dehydrated whole egg, dried apricots, and darkened orange juice (3,4,7,21). Attempts were therefore made to remove the pigments from browned bread crumb. Two methods were tried: 1) direct extraction of the pigment by means of various solvents; and 2) extraction after the crumb was first acetylated.

Methods. Direct extraction of the crumb (2.0 g.) by intermittent shaking with 50 ml. of various redistilled solvents—water, pyridine, methanol, ethanol, isobutanol (2-methyl-1-propanol), benzaldehyde, ethyl acetate, chloroform, toluene, and dioxane (1,4-dioxane)—at room temperature for 24 hours was tried. For the acetylation procedure, the crumb was first extracted with a benzene-ethanol mixture (60 + 40) to remove most of the lipids, after which it was dispersed and dried by the method used by Pacsu and Mullen in preparing starch for acetylation (17). Four grams of the extracted crumb were dispersed in a mixture of 60% pyridine and 40% water and this was allowed to stand for 20 hours. The water was then removed by distillation as an azeotropic mixture with pyridine in the ratio of 60% pyridine and 40% water. An excess of pyridine was added to ensure the removal of all the water while the crumb was allowed to remain in a dispersed state in the pyridine. To this dry pyridine suspension of crumb, 30 ml. of acetic anhydride were added and the mixture was allowed to react for a week at room temperature. To decompose the unreacted acetic anhydride, 95% ethanol was added to the reaction mixture and allowed to stand overnight. The solvents and excess reactants were removed by distillation at reduced pressure.

The acetylated crumb was then extracted with water and other solvents to ascertain whether the brown pigment could be dissolved.

To obtain an estimate of the amount of nitrogen present in the extracts, their hydrochloric acid content was adjusted to 20% and they were refluxed for 24 hours. The amino nitrogen of the hydrolyzed solution was determined with the Van Slyke apparatus and expressed as mg. nitrogen per g. dry matter.

Results. Direct extraction with any of the solvents tried was quite ineffective in removing the pigment from browned bread crumb. Such poor extraction was obtained that no quantitative measurements were undertaken. From visual examination of the extracts and the residual crumb, the efficiency of extraction with the various solvents was classified into the following groups:

1. Very little extraction (solvent not colored) – benzaldehyde, chloroform, dioxane, ethyl acetate, isobutanol, toluene, mixture of benzene and ethanol (1 + 1).
2. Slight extraction – various aqueous methanol solutions containing from 20 to 100% of methanol.
3. Some extraction – water,[9] pyridine.

Acetylation gave a brown reaction mixture with both browned and white bread crumb. Extraction of the acetylated crumb with water, after removal of the excess reactants and solvents, left a nearly-white residue. Analysis of the water extract after hydrolysis gave the following values for amino nitrogen:

Sample	*Nitrogen per g. dry matter*[a]		
	mg Expt. 1	*mg* Expt. 2	*mg* Mean
Nonbrowned crumb	6.7	9.4	8.0
Browned crumb (stored 17 days at 75°C.)	8.0	9.4	8.7

[a] Amino nitrogen values for each extract were determined in duplicate.

Despite marked differences in the extent of browning of the two samples, no significant differences in the nitrogen content of the water-soluble fractions of the acetylated crumb were found. The absorbance values for aqueous and chloroform extracts of acetylated "fresh" and browned crumb at wave lengths of 240 to 600 mμ were not materially different.

Discussion

These exploratory studies on canned bread reveal that rather drastic storage conditions in regard to temperature and time are necessary to produce significant browning. Moreover, crumb discoloration was quite extensive before any marked chemical changes

[9] A 4% solution of Penetrant 1, an alkyl aryl sulfonate (Arnold Hoffman and Co., Providence, R. I.) also failed to extract any appreciable quantity of the pigment, as judged by the color of the crumb.

were detected, and no clear-cut evidence was obtained of the nature of the reactions which were involved. The amino nitrogen content of the crumb increased as browning developed; these values paralleled the increases in soluble nitrogen, and when they were corrected for the changes in solubility, there was no significant change in free amino nitrogen. Moreover, the total reducing substances remained rather constant. The oxidizing reagent, potassium ferricyanide, employed in these experiments is not specific for reducing sugars but is also reduced by carbonyl compounds which result from the degradation of carbohydrates. The only specific evidence that the Maillard reaction may be involved in the discoloration of bread crumb was the decrease in lysine nitrogen, but this did not occur until browning was quite extensive.

Although the first step in the Maillard reaction is sugar-amine condensation, this may not be reflected by a loss in free amino groups as determined by formol titration. The N-glycoside is in equilibrium with the original sugar, amine, and water. Because of the reversible nature of the reaction, N-glycosides of amino acids do not show a loss of amino nitrogen. Taufel and Iwainsky (20) found that they had to use a copper-complexing method to determine the extent of amino acid combination with sugars.

The marked increases which occurred in titratable acidity and the decreases in pH during storage favor the postulation that organic acids may play a role in the browning of canned bread, as has been proposed as a result of studies with other foods. Although the composition of the brown pigment would provide some clue regarding the nature of the reactants which produce it, the attempts which are made to extract it were entirely unsuccessful.

These preliminary experiments indicate that investigations of the effect of formula variations on the browning of bread may be carried out at 75°C. In view of the very limited changes in the quantity of reducing substances during browning, it would seem advisable to study the nature of the carbonyl substances before and after browning occurred.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minnesota (1947).
2. Burton, W. G. Mashed potato powder. III. The high-temperature browning of mashed potato powder. J. Soc. Chem. Ind. **64:** 215–218 (1945).
3. Choi, R. P., Koncus, A. F., O'Malley, C. M., and Fairbanks, B. W. A proposed method for the determination of color of dry products of milk. J. Dairy Sci. **32:** 580–586 (1949).
4. Edwards, B. G., and Dutton, H. J. Changes in stored dried eggs. Role of phospholipides and aldehydes in discoloration. Ind. Eng. Chem. **37:** 1121–1122 (1945).

5. HAAS, VICTORIA A., and STADTMAN, E. R. Deterioration of dried fruits. Use of ion exchange resins to identify types of compounds involved in browning. Ind. Eng. Chem. **41:** 983–985 (1949).
6. HODGE, J. E. Chemistry of browning reactions in model systems. J. Agr. Food Chem. **1:** 928–943 (1953).
7. JOSLYN, M. A., and MARSH, G. L. Browning of orange juice. Ind. Eng. Chem. **27:** 186–189 (1935).
8. LARSEN, R. A., KOCH, R. B., and MCMULLEN, J. J. The browning of canned bread crumb. Food Technol. **8:** 355–357 (1954).
9. LAUFER, S. The factors influencing color of beer and ale. Am. Brewer **74:** 20–24 (1941).
10. LEA, C. H. The role of amino-acids in the deterioration of food: the browning reaction. Chem. and Ind. (London) **9:** 155–158 (1950).
11. LEA, C. H., and HANNAN, R. S. Studies of the reaction between proteins and reducing sugars in the "dry" state. I. The effect of activity of water, of pH and of temperature on the primary reaction between casein and glucose. Biochim. et Biophys. Acta **3:** 313–325 (1949).
12. LEA, C. H., and HANNAN, R. S. Studies of the reaction between proteins and reducing sugars in the "dry" state. III. Nature of the protein groups reacting. Biochim. et Biophys. Acta **5:** 433–454 (1950).
13. LEA, C. H., and HANNAN, R. S. Biochemical and nutritional significance of the reaction between proteins and reducing sugars. Nature **165:** 438–439 (1950).
14. LEWIS, V. M., ESSELEN, W. B., JR., and FELLERS, C. R. Non-enzymatic browning of foodstuffs. Nitrogen-free carboxylic acids in the browning reaction. Ind. Eng. Chem. **41:** 2591–2594 (1949).
15. MA, T. S., and ZUAZAGA, G. Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. Ind. Eng. Chem., Anal. Ed. **14:** 280–282 (1942).
16. MAILLARD, L. C. Chimie Organique. Action des acides amines sur les sucres; formation des melanoidines par boie methodique. Compt. rend. **154:** 66–68 (1912).
17. PASCU, E., and MULLEN, J. W. Procedure for the preparation of certain derivatives of starch. J. Am. Chem. Soc. **63:** 1487–1488 (1941).
18. PEPKOWITZ, L. P., and SHIVE, J. W. Kjeldahl nitrogen determination. A rapid wet-digestion micromethod. Ind. Eng. Chem., Anal. Ed. **14:** 914–916 (1942).
19. STEWART, G. F., and KLINE, R. W. Factors influencing the rate of deterioration in dried egg albumin. Ind. Eng. Chem. **40:** 916–919 (1948).
20. TÄUFEL, T., and IWAINSKY, H. Analytische und chromatographische Studien zur "Maillard-Reaktion". Biochem. Z. **323:** 299–308 (1952).
21. WEAST, C. A., and MACKINNEY, G. Nonenzymatic darkening of fruits and fruit products. Ind. Eng. Chem. **33:** 1408–1412 (1941).
22. ZITTLE, C. A., and ELDRED, N. R. Determination of l-lysine with a specific decarboxylase. J. Biol. Chem. **156:** 401–409 (1944).

STUDIES ON ACCELERATED BROWNING IN STARCH PASTES CONTAINING VARIOUS BREAD INGREDIENTS[1]

RUSSELL J. STENBERG[2] AND W. F. GEDDES

ABSTRACT

Wheat starch pastes (10%) with and without single and multiple additions of wheat gluten, glucose, lactose, shortening, and nonfat dry milk, prepared at pH values of 4.4, 5.4, and 6.4, were stored at 75°C. for 25 days. The sugars were the principal ingredients which caused browning, as measured by reflectance of the dried pastes at 600 mμ. Browning was accompanied by increases in reducing substances, even in pastes made without sugars. Aqueous ethanol extracts of the stored pastes exhibited absorption maxima at approximately 275 mμ, a region where carbonyl compounds show strong absorption. Chromatographic separation on magnesium sulfate of the 2,4-dinitrophenylhydrazones prepared from ethanol extracts of a browned paste made with wheat starch, gluten, and sucrose at pH 6.4 yielded 13 "nonacid" and 17 "acid" carbonyl derivatives. The absorbance of these derivatives did not correspond to that of the corresponding hydrazones of 5-hydroxymethylfurfural or levulinic acid.

Exploratory studies conducted by the authors with canned bread revealed that rather drastic conditions of time and temperature were necessary to produce any appreciable browning (16). The development of a brown color was paralleled by increases in total soluble nitrogen, amino nitrogen, and titratable acidity, whereas reducing substances remained essentially constant. Lysine N, however, decreased with an increase in storage time at the highest storage temperatures employed (50° to 75°C.), but browning was already quite extensive before any decrease was noted.

In the light of these preliminary studies, it was decided to investigate the effects of different bread ingredients on the browning phenomena by using model systems containing various combinations of ingredients stored at 75°C. Since 5-hydroxymethylfurfural is a recognized intermediate in browning (6,7) and has been reported to increase in bread in proportion to the amount of sugar used in the formula (9), several of the systems were examined for the presence of

[1] Manuscript received January 13, 1960. Contribution from the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota. Paper No. 4210 of the Scientific Journal series, Minnesota Agricultural Experiment Station. The data in this paper represent part of a thesis presented to the Graduate School of the University of Minnesota by Russell J. Stenberg in partial fulfillment of the requirements for the Ph.D. degree, May 1950.

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number 1079 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

[2] Present address: Northern Utilization Research and Development Division, Agricultural Research Service, USDA, Peoria, Illinois.

carbonyl compounds. The experiments were carried out with wheat starch pastes containing one or more of the other ingredients used in making bread.

Materials and Methods

The following materials were used:

Wheat starch. A specially prepared product (General Mills Research Laboratories, Minneapolis, Minn.) washed from a hard winter wheat flour dough, without the use of sulfur dioxide. This starch was processed to contain not more than 0.4% protein (N × 5.7, dry basis).

Wheat gluten. Prepared by washing starch from a spring wheat flour dough. The gluten in thin sheets was dried at room temperature (about 23°C.) in moving air and then ground in a semi-micro Wiley mill to pass a 40-mesh sieve.

Sucrose, reagent grade (General Chemical Co.)

D-glucose—C.P. (Mallinckrodt)

Lactose (U.S.P.)

Nonfat dry milk, specially prepared for breadmaking by the spray process.

Shortening: leaf lard, kettle-rendered.

Preparation and Storage of Starch Paste Mixtures. Pastes were prepared by stirring 10 g. of starch and one or more of the other ingredients with 90 ml. of distilled water or buffer solution for 25 minutes at 75°C. Biphthalate buffers (0.05*M*) were used for pH values of 4.4 and 5.4 and phosphate buffer (0.05*M*) for pH 6.4.

With the exception of nonfat dry milk, where 0.25 g. was used, 0.5 g. of each additional ingredient was employed in preparing the pastes. They were stored in 250-ml. Erlenmeyer flasks for 25 days in a circulating-air oven maintained at 75°C.

Methods of Analysis. At the beginning and end of the storage period, a portion of each paste was frozen, crushed, and lyophilized. The dried material containing 4 to 5% moisture was ground in a semi-micro Wiley mill fitted with a 20-mesh screen. The color of the ground product was measured at 600 $m\mu$ by means of a Beckman Model DU spectrophotometer with a reflectance attachment, using a freshly scraped block of magnesium carbonate as a reference standard. All other analytical determinations were made on the pastes without drying them.

Reducing and nonreducing substances were determined by a modification of the procedures for reducing and nonreducing sugars described in *Cereal Laboratory Methods* (1). The modification consisted of stirring 25 g. of the paste with 10 ml. of 95% ethanol and conduct-

ing the sugar analysis on a 10-ml. aliquot. Results were expressed as the ml. of 0.1*N* ferricyanide reduced.

Spectrophotometric measurements in the ultraviolet region were made on solutions prepared from some of the pastes. The well-mixed paste (25 g.) in a 125-ml. Erlenmeyer flask was extracted with 35 ml. of 95% ethanol which had been purified by distillation over potassium hydroxide and zinc dust. The extract was clarified by filtration and its absorbance determined in a Beckman Model DU spectrophotometer at wave lengths from 220 to 320 mμ. For comparative purposes, the absorbances of levulinic acid and 5-hydroxymethylfurfural in the ultraviolet region were also determined. Levulinic acid was purified by redistilling twice under reduced pressure in an atmosphere of nitrogen. The resulting colorless product crystallized readily at 7°C., melted at 31°C. with refractive index $n_D^{27} = 1.4388$. The method described by Haworth and Jones (5) was used to prepare 5-hydroxymethylfurfural. The product was distilled twice under high vacuum to yield a light yellow liquid which crystallized readily at 0°C. (refractive index $n_D^{25} = 1.5602$).

Separation of Carbonyl Compounds. For the isolation and attempted characterization of carbonyl compounds, a browned paste made with wheat starch, wheat gluten, and sucrose buffered at pH 6.4 and stored for 25 days at 75°C. was employed. The paste (400 g.) was extracted with 400 ml. of purified 95% ethanol and the extract removed by filtration. A second filtrate was obtained by extracting the residue with 100 ml. of 95% ethanol for 1 hour. The filtrates were combined and concentrated to about 200 ml. by heating at 35°C. under reduced pressure, after which 10 g. of sodium bicarbonate were added to neutralize any organic acids which were present. Continuous extraction with redistilled diethyl ether in a modified Soxhlet apparatus for 6 hours removed aldehydes and ketones which did not contain acidic groups ("nonacid" carbonyl compounds). The residual solution was then acidified with hydrochloric acid equivalent to the sodium bicarbonate previously added, and continuously extracted with redistilled diethyl ether to remove the "acid" carbonyl compounds. Each of the ether solutions was concentrated at room temperature under reduced pressure to about 25 ml. Water (10 ml.) was then added and the solutions were reduced to a final volume of 15 to 20 ml.

To prepare the 2,4-dinitrophenylhydrazones of the carbonyl compounds, 2,4-dinitrophenylhydrazine hydrochloride was dissolved in 2*N* hydrochloric acid according to the directions of Brady and Elsmie (2). Each solution of carbonyl compounds was added to 200 ml. of the freshly prepared and filtered reagent and after 3.0 hours the reaction

mixture was extracted three times with redistilled diethyl ether. The extracts were combined and the ether removed under reduced pressure to yield a mixture of any 2,4-dinitrophenylhydrazones which were present and any unreacted reagent which may have been extracted.

The chromatographic technique used in separating those hydrazones was patterned after the method employed by Stadtman (15) in the separation of similar derivatives obtained in studies on dried apricots. After development of the chromatograms, the hydrazones were extracted from each band with purified diethyl ether.

The eluate from each band was evaporated to dryness under reduced pressure. To remove occluded benzene, the residue was dissolved in purified 95% ethanol and once more evaporated to dryness; this process was repeated three times.

The 2,4-dinitrophenylhydrazones of 5-hydroxymethylfurfural melted at 200°C., as compared with 200.3°C. reported by Wahhab (18); the levulinic acid derivative melted at 206°C., the same as reported in the literature (8).

The absorbance of solutions of the 2,4-dinitrophenylhydrazones isolated from the browned pastes and the corresponding derivatives of the two reference compounds was determined with the Beckman DU spectrophotometer at wave lengths from 225 to 450 mμ.

Bacteriological examinations were made on ten samples of the pastes which had been stored by plating on a dextrose-tryptone agar. A fluid thioglycollate medium which is capable of supporting many types of aerobic and anaerobic organisms was also inoculated with successive dilutions of the pastes[3].

Results

Bacteriological Tests. The plate counts and dilution tests carried out on several of the stored samples gave no evidence of bacterial contamination. Bacteria were, therefore, not involved in the chemical changes which occurred in the pastes during storage.

Effect of Ingredients and pH on Color Formation in Pastes during Storage. The reflectance data recorded in Table I do not show any consistent relation between pH and browning over the range investigated. The pH values of the buffered pastes did not change during storage, whereas the acidity of an unbuffered starch paste fell from pH 6.1 immediately after preparation to pH 3.4 after being held for 25 days at 75°C. Studies on the browning of several food products have revealed that the rate of color development decreases with a decrease

[3] The bacteriological tests were carried out by Dr. J. J. Jezeski, Department of Dairy Husbandry, University of Minnesota.

in pH (4,11,17). However, the effect of pH over the range employed with these pastes was slight and inconsistent.

Comparisons of the reflectance changes at corresponding pH values in the different pastes clearly show that the sugars were the major contributors to browning. When added to starch or to mixtures containing starch, gluten, shortening, and nonfat dry milk, sucrose caused more discoloration than glucose. Unfortunately, no pastes were prepared to determine the effect of D-fructose formed along with glucose by the inversion of sucrose. The contribution of lactose to browning appeared to be less than that of the other two sugars.

The effects of the other ingredients on the formation of brown pigments varied, depending on the nature of the other substances

TABLE I

EFFECT OF INGREDIENTS AND PH OF PASTES ON COLOR FORMATION DURING STORAGE FOR 25 DAYS AT 75°C.

COMPOSITION OF PASTE	REFLECTANCE		
	pH 4.4	pH 5.4	pH 6.4
Starch only (not buffered, not stored), 0.91[a]	...	...	...
Starch only (not buffered, stored), 0.92[b]	...	...	...
Starch only	0.71	0.72	0.87
Starch + gluten (control, not buffered, not stored), 0.90[c]	...	...	...
Starch + gluten	.68	.85	.81
Flour	.72	.72	.68
Starch + sucrose	.70	.69	.63
Starch + glucose	.76	.71	.68
Starch + lactose	.69	.74	.72
Starch + NFDM[d] (control, not stored)	.90	..	.88
Starch + NFDM	.71	.74	.82
Starch + shortening (control, not stored)	.86	..	.84
Starch + shortening	.73	.76	.84
Starch + gluten + sucrose	.64	.67	.73
Starch + gluten + glucose	.66	.65	.67
Starch + gluten + lactose	.80	.75	.72
Starch + gluten + NFDM	.80	.80	.77
Starch + gluten + shortening	.75	.77	.75
Starch + shortening + sucrose	.61	.64	.74
Starch + shortening + glucose	.77	.80	.82
Starch + glucose + NFDM	.70	.63	.72
Starch + gluten + shortening + sucrose	.65	.76	.74
Starch + gluten + shortening + NFDM	.65	.77	.84
Starch + shortening + sucrose + NFDM	.73	.77	.83
Starch + gluten + sucrose + NFDM	.62	.70	.78
Starch + gluten + shortening + NFDM + sucrose	.62	.67	.73
Starch + gluten + shortening + NFDM + glucose	.63	.75	.79
Starch + gluten + shortening + NFDM + lactose	0.79	0.81	0.78

[a] This unbuffered paste immediately after preparation had a pH of 6.0.
[b] This unbuffered paste had a pH of 3.4 after 25 days' storage.
[c] This unbuffered paste had a pH of 6.1 immediately after preparation.
[d] Nonfat dry milk.

which were present. Gluten caused an increase in coloration when added to mixtures of starch and sucrose or of starch and glucose. In other pastes, gluten appeared to play a less important role. Nonfat dry milk apparently retarded browning when present in a paste containing starch, shortening, and sucrose. Shortening also retarded coloration in mixtures of starch and glucose and of starch, gluten, and sucrose. In other pastes, shortening and nonfat dry milk had no effect on the development of color during storage.

These data on the marked influence of the sugars on the browning of the pastes have been confirmed by the subsequent work of Larsen *et al.* on the browning of canned bread prepared by formulas varying in sugar content (10).

Effect of Paste Ingredients on Changes in Reducing and Nonreducing Substances during Storage. Reducing and nonreducing substances were determined in pastes stored at pH 5.4, the active acidity which was the closest to that found in canned bread prepared by the original formula.

The results in Table II show that increases in reducing substances occurred even in those pastes made with starch alone, although the

TABLE II

EFFECT OF COMPOSITION OF PASTE ON AMOUNT OF MATERIALS REDUCED BY ALKALINE FERRICYANIDE DURING STORAGE AT pH 5.4 FOR 25 DAYS AT 75°C.

COMPOSITION OF PASTE	REDUCING SUBSTANCES[a]		
	Stored	Control	Gain
Starch only	0.6	0.1	0.5
Starch + gluten	1.4	0.8	0.6
Flour	4.3	2.1	2.2
Starch + sucrose	3.3	0.1	3.2
Starch + glucose	7.4	6.2	1.2
Starch + lactose	5.6	5.0	0.6
Starch + NFDM[b]	2.2	1.4	0.8
Starch + shortening	1.0	0.1	0.9
Starch + gluten + sucrose	3.9	0.5	3.4
Starch + gluten + glucose	7.6	5.6	2.0
Starch + gluten + lactose	6.4	3.1	3.3
Starch + gluten + NFDM	2.1	1.6	0.5
Starch + gluten + shortening	1.0	0.3	0.7
Starch + shortening + sucrose	4.4	0.1	4.3
Starch + shortening + glucose	6.6	6.1	0.5
Starch + NFDM + glucose	8.1	7.6	0.5
Starch + gluten + shortening + sucrose	3.1	0.2	2.9
Starch + gluten + shortening + NFDM	2.6	1.7	0.9
Starch + shortening + NFDM + sucrose	4.8	1.3	3.5
Starch + gluten + NFDM + sucrose	4.3	1.5	2.8
Starch + gluten + shortening + NFDM + sucrose	4.7	1.5	3.2
Starch + gluten + shortening + NFDM + glucose	8.3	7.5	0.8
Starch + gluten + shortening + NFDM + lactose	6.0	6.0	0.0

[a] Reducing substances are expressed as ml. 0.1*N* ferricyanide reduced per 25 g. paste.
[b] Nonfat dry milk.

reducing power was further increased by the addition of other ingredients, particularly the sugars.

The reducing and nonreducing substances in pastes to which sucrose was added are recorded in Table III. These determinations were carried out on a different series of pastes from those for which analyses are given in Table II, and the differences in the gain in reducing sugars for comparable samples in Tables II and III give a measure of the reproducibility of the experiments. The gain in reducing substances during storage was substantially greater than the loss in nonreducing substances, so that reducing substances have arisen from sources other than sucrose. When sucrose solution was stored at pH 5.4, however, the increase in reducing substances equaled the decrease in nonreducing materials.

TABLE III

CHANGES IN REDUCING AND NONREDUCING SUBSTANCES FOUND IN WHEAT STARCH PASTES CONTAINING SUCROSE AND OTHER BREAD INGREDIENTS AFTER STORAGE AT PH 5.4 FOR 25 DAYS AT 75°C.

COMPOSITION OF PASTE	REDUCING SUBSTANCES[a]		GAIN IN REDUCING SUBSTANCE[a]	NONREDUCING SUGARS[a]		LOSS IN NON-REDUCING SUGARS[a]
	Stored	Control		Stored	Control	
Starch + sucrose	2.4	0.2	2.2	3.4	4.7	1.3
Starch + sucrose + gluten	3.3	0.4	2.9	3.0	4.8	1.8
Starch + sucrose + shortening	2.6	0.2	2.4	3.0	3.6	0.6
Starch + sucrose + shortening + gluten	2.9	0.3	2.6	2.8	4.6	1.8
Starch + sucrose + shortening + NFDM[b]	4.1	1.4	2.7	3.1	4.9	1.8
Starch + sucrose + gluten + NFDM	2.6	1.7	0.9	4.0	4.7	0.7
Starch + sucrose + gluten + shortening + NFDM	3.8	1.7	2.1	3.3	4.4	1.1
Sucrose solution (not a paste)	2.9	0.0	2.9	3.1	6.4	3.3

[a] Expressed as ml. of 0.1*N* ferricyanide per 25 g. paste.
[b] Nonfat dry milk.

Spectrophotometric Analyses of Aqueous Ethanol Extracts of Wheat Starch Pastes. Absorbance values determined on 50% ethanol extracts of representative pastes which were made with phosphate buffer (pH 6.4) are recorded in Figs. 1 and 2[4]. These data show that

[4] The biphthalate ion showed strong absorption in the same spectral region as the carbonyl compounds, and hence curves were not made for pastes containing this buffering agent. Absorption of the alcoholic extracts was confined essentially to the ultraviolet region, and the curves have therefore been plotted only for the region from 215 to 320 mμ.

The effect of ethanol concentration on the absorption characteristics of an extract prepared from a wheat starch paste containing gluten and sucrose stored at pH 6.4 for 25 days was investigated. One aliquot was diluted with 50% and another with 95% ethanol. The increase in ethanol concentration had no influence on the character of the absorption curve.

In another experiment the effect of diethyl ether extraction was studied. Twenty milliliters of the undiluted extract from a stored paste which contained starch, gluten, and glucose was further extracted with diethyl ether for 6 hours. The ether was removed by distillation and the residue made up to 200 ml. with 50% ethanol. Extraction with diethyl ether shifted the maximum absorption from 273 to 277.5 mμ and a decrease in the quantity of absorbing material also resulted.

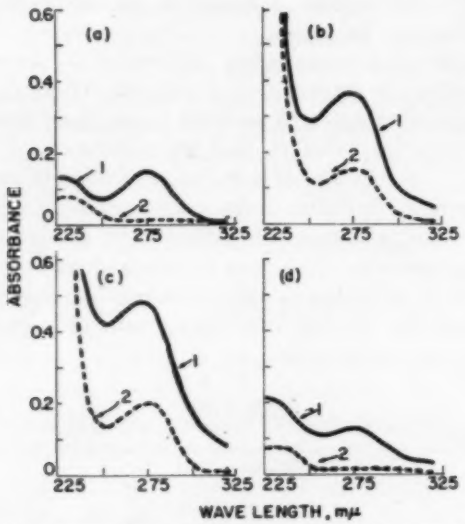


Fig. 1. Ultraviolet absorption curves for 50% ethanol extracts of starch pastes of various composition before and after storage for 25 days at 75°C. Curve 1, after storage; curve 2, before storage; (a) wheat starch only; (b) wheat starch + wheat gluten; (c) wheat flour; (d) wheat starch + sucrose.

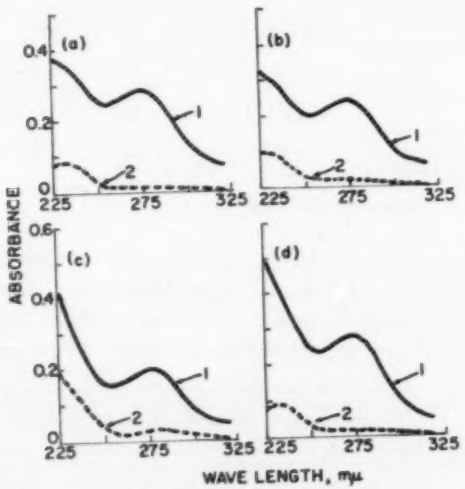


Fig. 2. Ultraviolet absorption curves for 50% ethanol extracts of starch pastes of various composition before and after storage for 25 days at 75°C. Curve 1, after storage; curve 2, before storage; (a) wheat starch + glucose; (b) wheat starch + lactose; (c) wheat starch + nonfat dry milk; (d) wheat starch + shortening.

a substance or group of substances which exhibited an absorption maximum at approximately 275 mμ was produced in each of the pastes during storage.

The increases in absorbance at 275 mμ during storage of the various pastes for 25 days at 75°C. are recorded in Table IV. A paste made with wheat flour showed a great increase in absorbance on storage. The presence of sugars in the pastes is associated with a large increase in absorbance; glucose caused the greatest increase, followed by lactose and sucrose in the order named. Surprisingly, nonfat dry milk which contains about 50% lactose, when present in a paste, depressed the change in absorbance during storage.

Comparison of Absorption Curves for Extracts of Starch Pastes with 5-hydroxymethylfurfural and Levulinic Acid. The absorption curves and constants for 5-hydroxymethylfurfural dissolved in water and in absolute ethanol are given in Fig. 3. Changing the solvent from water to absolute ethanol shifted the maximum absorption from 283 to 280 mμ. When the solvent was 50% ethanol the spectral curve followed that of the water solution very closely.

The absorption curves for pure levulinic acid in water, and in 50%

TABLE IV

EFFECT OF COMPOSITION ON THE INCREASE IN ABSORBANCE OF EXTRACTS OF WHEAT STARCH PASTES AT pH 6.4 DURING STORAGE AT 75°C. FOR 25 DAYS

COMPOSITION OF PASTE	INCREASE IN ABSORBANCE[a] AT 275mμ
Starch only	0.14
Starch + gluten	.22
Flour	.28
Starch + sucrose	.12
Starch + glucose	.27
Starch + lactose	.22
Starch + NFDM[b]	.17
Starch + shortening	.27
Starch + gluten + sucrose	.31
Starch + gluten + glucose	.38
Starch + gluten + lactose	.31
Starch + gluten + NFDM	.16
Starch + gluten + shortening	.17
Starch + shortening + sucrose	.21
Starch + shortening + glucose	.21
Starch + glucose + NFDM	.16
Starch + gluten + shortening + sucrose	.23
Starch + gluten + shortening + NFDM	.17
Starch + sucrose + shortening + NFDM	.16
Starch + gluten + sucrose + NFDM	.17
Starch + gluten + shortening + NFDM + sucrose	.18
Starch + gluten + shortening + NFDM + glucose	.22
Starch + gluten + shortening + NFDM + lactose	0.24

[a] The increases are the difference in absorbance of extracts of pastes before and after storage.
[b] Nonfat dry milk.

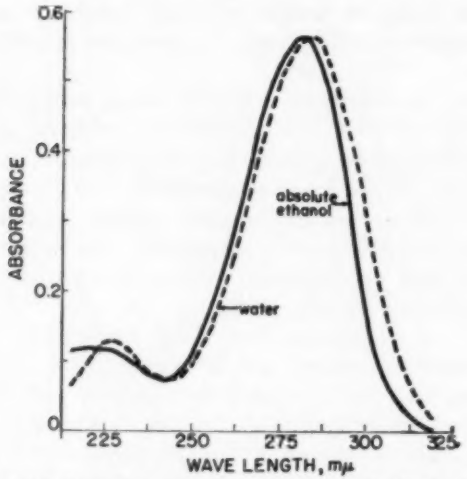


Fig. 3. Ultraviolet absorption curves for 5-hydroxymethylfurfural in aqueous and absolute ethanol solutions.

Absorption constants in water:

Max., 283 mμ	Min. 242.5 mμ	Max. 227 mμ
E = 15670	E = 2175	E = 3600

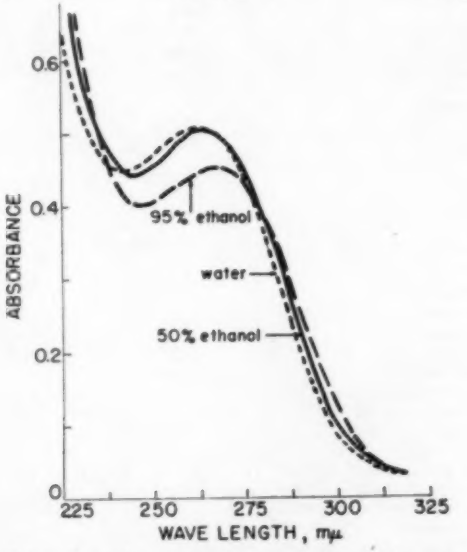


Fig. 4. Ultraviolet absorption curves for levulinic acid in water, 50% and 95% aqueous ethanol.

Absorption constants in water:

Min. 241 mμ	C = 0.0244 moles/liter	Max. 262.5 mμ
E = 24.7		E = 27.8

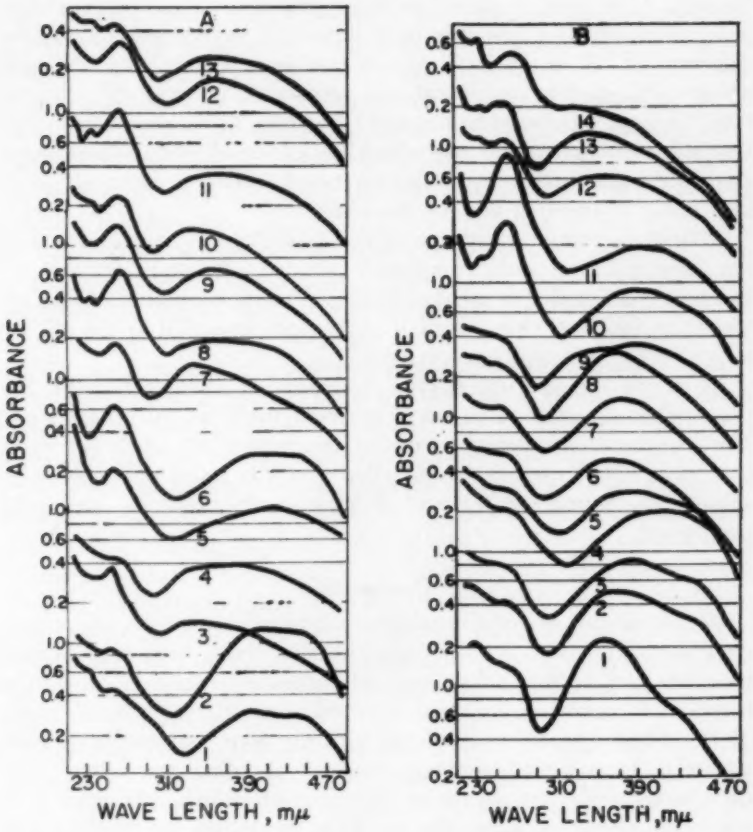


Fig. 5. Absorption spectra of 2,4-dinitrophenylhydrazones of (A) "nonacid" and (B) "acid" carbonyl compounds extracted from a paste prepared from wheat starch, wheat gluten, and sucrose after storage for 25 days at 75°C. Curves are numbered in order of increasing affinity of the derivatives on magesium sulfate.

and 95% ethanol are given in Fig. 4. When absorption data were calculated for a mixture of $2.812 \times 10^{-5}M$ hydroxymethylfurfural and $1.219 \times 10^{-2}M$ levulinic acid, a spectral curve was obtained which was very similar to that for an extract of a paste prepared from starch, gluten, and sucrose. This indicates that both of these carbonyl compounds may be present in the pastes after storage.

Separation and Attempted Characterization of Carbonyl Compounds in Stored Pastes. The method employed for separating the "acid" carbonyl compounds from aldehydes and ketones which do not possess acidic groups was first applied to a known mixture of

levulinic acid and furfural in aqueous solution[5]. The respective maxima of the two fractions were separated by at least 10 mμ. Assuming that all of the furfural was extracted by diethyl ether, only about 40% of this compound was recovered, whereas 90% of the levulinic acid was found in the water fraction. These results indicated that the carbonyl compounds which are extracted from wheat pastes could be separated into acid and nonacid fractions, although losses may occur in carrying out the procedure.

Chromatographic separation of the 2,4-dinitrophenylhydrazones of the "nonacid" carbonyl compounds yielded 13 bands, whereas 17 bands were obtained from the "acid" carbonyl compounds. The absorbances for these two groups of hydrazones from 220 to 490 mμ are shown in Fig. 5, A and B respectively.

None of the spectral curves corresponded to that for the 2,4-dinitrophenylhydrazone of 5-hydroxymethylfurfural. Although curve No. 1 of the acid carbonyl derivatives resembled that of the levulinic acid compound, the absorption affinity of the latter on magnesium sulfate was much greater and it cannot, therefore, be the same compound.

Discussion

Measurements of color development in the pastes show that sugars were the major, but not the only, contributors to browning. Several reactions can occur. They may decompose to furfurals or similar compounds involving the sugars containing an active carbonyl group (5,18). These may either condense with nitrogen compounds or polymerize to form brown, resinous materials. Another possibility is the decomposition of the sugars to organic acids which may react with sugars, nitrogenous materials, or with themselves to produce pigments. Because the pastes, with one exception, were well buffered, any increases in the quantity of acids could not be detected through pH changes; however, the fact that the pH of an unbuffered starch paste fell from pH 6.0 to 3.4 showed that organic acids were produced.

The increase in substances which reduce ferricyanide was associated with the development of browning in the pastes. This increase was not due solely to the hydrolysis of sucrose, since pastes which did not contain this sugar browned and showed increases in reducing substances. Hydrolysis of starch yields maltose and glucose which could decompose to produce such reducing substances as dihydroxyacetone

[5] Sodium bicarbonate was added to form the sodium salt of levulinic acid which is insoluble in diethyl ether. The solution was then extracted four times with diethyl ether, to remove the furfural. After evaporation of the ether at reduced pressure, the extracted material was made up to volume in water and the absorbance of both fractions determined. Sodium bicarbonate had no effect upon absorbance between 260 and 320 mμ.

and glyceraldehyde, which have been postulated as intermediates in the browning reaction by Speck and Ball (14). Reducing substances which may arise from the breakdown of sugars include glyoxal, methyl glyoxal, diacetyl, acetol acetoin, and the furfurals, all of which may be intermediates in color formation. The furfurals, especially, have received attention as possible precursors of the dark pigments in dried fruit (12,13), and 5-hydroxymethylfurfural has been reported in bread (9).

The marked absorbance of extracts of the pastes at about 275 $m\mu$ is characteristic of the carbonyl group, and the chromatographic separation of the 2,4-dinitrophenylhydrazones indicated the presence of 13 carbonyl compounds in the "nonacid" group and 17 in the "acid" group, despite the probable loss of the more volatile carbonyl compounds such as acetaldehyde due to the method of separation employed. It seems unlikely, however, that 30 different hydrazones were isolated. There is a possibility that the separation into the two groups was incomplete, so that some of the same derivatives may be present in both; also, it has been shown that 2,4-dinitrophenylhydrazones which exist as cis, trans isomers may be partially separated by chromatographic methods (20). In the present experiments, the 2,4-dinitrophenylhydrazone of 5-hydroxymethylfurfural yielded two bands of nearly equal color intensity and the same absorption when chromatographed on a magnesium sulfate column. Tautomerization of the 2,4-dinitrophenylhydrazones may also be a source of some of the many compounds observed.

The distribution of the absorption maxima in the range between 330 and 410 $m\mu$ (phenylhydrazine absorption) indicates that more than one class of derivative is present. It has been shown that the position of the absorption band attributed to the phenylhydrazine part of the molecule is dependent upon the extent of conjugated unsaturation and, to a lesser degree, upon the amount of alkyl substitution present in the parent carbonyl compound (21,22). General classifications of the 2,4-dinitrophenylhydrazones have been made on this basis. Other characteristics, besides the degree of unsaturation in the parent carbonyl compound, however, may alter the nature of the "phenylhydrazine" absorption; thus λ max. increases considerably for the bis-2,4-dinitrophenylhydrazones of the alpha-diketones where conjugation occurs between the two hydrazone systems (22). From the phenylhydrazine absorption of the derivatives in these experiments it was not possible to arrive at any classification of the parent carbonyl compounds, but it may be concluded that they varied in nature.

The absorption maximum at 260 $m\mu$ exhibited by all the deriva-

tives is also shown by 2,4-dinitrophenylhydrazine itself. The fact that derivatives which were most strongly adsorbed by magnesium sulfate had higher ^{E}Max. values in the range of 220 to 300 mμ than the ^{E}Max. value of the phenylhydrazine portion of the molecule cannot be explained. None of the 50 compounds studied by Braude and Jones (3) corresponded to those found in the present studies. The fact that none of the hydrazones corresponded to those of 5-hydroxymethylfurfural or levulinic acid does not mean that they were not formed. These, and other very reactive carbonyl compounds, could have reacted further to form the brown pigments, leaving the less reactive carbonyls free to be isolated as hydrazones.

In a personal communication Hodge[6] has discussed these findings in the light of his investigations of the chemistry of browning reactions (6,7). The first step in the Maillard reaction is sugar-amine condensation. With secondary amines, the amine is then eliminated from carbon 1 of the sugar after 1,2- and 2,3-enolization of the sugar moiety. This generates a terminal methyl group at carbon 1 and releases the amine (amino acid) for further reaction. After this sequence of reactions a methyl-carbonyl-carbonyl arrangement exists on carbons 1, 2, and 3 of the erstwhile sugar, and this d-dicarbonyl compound is considered to be a critical intermediate for browning. It undergoes fission and dehydration without fission to give polymerizing unsaturated carbonyl compounds. Presumably some of these compounds have been isolated as 2,4-dinitrophenylhydrazones in the present study.

The derivatives of the unknown carbonyl compounds which were separated in these experiments cannot be identified until the absorption spectra of the 2,4-dinitrophenylhydrazones of a series of reference carbonyl compounds, which include all those which are present in the pastes, have been studied and classified[7].

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minnesota (1947).
2. Brady, O. L., and Elsmie, Gladys V. The use of 2:4-dinitrophenylhydrazine as a reagent for aldehydes and ketones. Analyst **51**: 77–78 (1926).
3. Braude, E. A., and Jones, E. R. H. Studies in light absorption. Part II. 2,4-dinitrophenylhydrazones. J. Chem. Soc. **1945**: 498–503.
4. Burton, W. G. Mashed potato powder. III. The high-temperature browning of mashed potato powder. J. Soc. Chem. Ind. **64**: 215–218 (1945).
5. Haworth, W. N., and Jones, W. G. M. The conversion of sucrose into furan compounds. Part 1. 5-Hydroxymethyl furfuraldehyde and some derivatives. J. Chem. Soc. **1944**: 667–670.

[6] J. E. Hodge, Northern Utilization Research and Development Division, Peoria, Illinois.

[7] Recently, Wiseblatt (19) has succeeded in identifying several of the carbonyl compounds distilled from bread under high vacuum by the formation, chromatographic separation, and characterization of their 2,4-dinitrophenylhydrazones by comparison with authentic hydrazones of known carbonyl compounds which have been prepared since the studies in this paper were undertaken.

6. HODGE, J. E. Chemistry of browning reactions in model systems. J. Agr. Food Chem. **1:** 928–941 (1953).
7. HODGE, J. E. The Amadori rearrangement. Advances in carbohydrate chemistry, vol. 10, pp. 169–205 (1955).
8. HUNTRESS, E. H., and MULLIKEN, S. P. Identification of pure organic compounds. Wiley: New York (1941).
9. KRETOVICH, V. L., and TOKAREVA, R. R. Content of hydroxymethylfurfural in bread and hops. Doklady Akad. Nauk. S.S.S.R. **74:** 533–535 (1950); Chem. Abstr. **45:** 1695e (1951).
10. LARSEN, R. A., KOCH, R. B., and MCMULLEN, J. J. The browning of canned bread crumb. Food Technol. **8:** 355–357 (1954).
11. LAUFER, S. The factors influencing color of beer and ale. Am. Brewer **74:** 20 (1941).
12. MACKINNEY, G., and TEMMER, ODETTE. The deterioration of dried fruit. IV. Spectrophotometric and polarographic studies. J. Am. Chem. Soc. **70:** 3586–3590 (1948).
13. MACMILLAN, D., and MELVIN, E. H. Ultraviolet light-absorption of alkali-treated solutions of carbohydrates. Science **107:** 447–449 (1948).
14. SPECK, J. C., JR., and BALL, C. D. A study of some chemical factors involved in the browning of certain food constituents. Committee on Food Research, Office of the Quartermaster General, File No. B-907, Report No. 5 (Dec. 1, 1945 to June 30, 1946).
15. STADTMAN, F. H. The chemical deterioration of dried fruit during storage. III. Chromatographic separation of carbonyl compounds as 2,4-dinitrophenylhydrazones. J. Am. Chem. Soc. **70:** 3583–3586 (1948).
16. STENBERG, R. J., and GEDDES, W. F. Some chemical changes which accompany the browning of canned bread during storage. Cereal Chem. **37:** 614–622 (1960).
17. STEWART, G. F., and KLINE, R. W. Factors influencing the rate of deterioration in dried egg albumin. Ind. Eng. Chem. **40:** 916–919 (1948).
18. WAHHAB, A. Deterioration of dried fruits. II. Identification of furfurals. J. Am. Chem. Soc. **70:** 3580–3582 (1948).
19. WISEBLATT, L., and KOHN, F. E. Some volatile aromatic compounds in fresh bread. Cereal Chem. **37:** 55–66 (1960).
20. ZERBAN, F. W. Bibliography of sugar-protein reactions with special reference to the sucrose industry. Committee on Food Research, Office of the Quartermaster General, File No. BB-Special, Report No. 2 (July to Sept., 1945).
21. ZERBAN, F. W. Caramel substances and melanoidins in the sucrose industry. Committee on Food Research, Office of the Quartermaster General, File No. BB-Special, Report No. 3 (July to Sept., 1945).
22. ZERBAN, F. W. Coloring matters formed from sugars in the absence of amino compounds. Committee on Food Research, Office of the Quartermaster General, File No. BB-Special, Report No. 1 (July to Sept., 1945).

IDENTIFICATION OF CARBONYL COMPOUNDS IN AN ETHANOL EXTRACT OF FRESH WHITE BREAD[1]

H. Ng, D. J. Reed[2], and J. W. Pence

ABSTRACT

A number of carbonyl compounds were isolated from an ethanol extract of fresh white bread by the formation of their 2,4-dinitrophenylhydrazone derivatives. Through these derivatives five carbonyl compounds were identified by conventional analytical procedures: acetaldehyde, ethyl pyruvate, furfural, hexanal, and 2-methylbutanal. Three additional carbonyl compounds were identified by means of paper chromatography in three varied systems and by ultraviolet spectrum data; these compounds are acetone, methyl ethyl ketone, and formaldehyde. Isobutyraldehyde and n-valeraldehyde, which were found in baking-bread vapors, appeared to be absent in the ethanol extract of bread.

The flavor and aroma of freshly baked bread deteriorate rapidly, resulting in a much less appealing product if distribution from baking centers requires more than a few hours. If fresh flavor could be stabilized, a better product could be made available to consumers. A major approach to the problems of improvement and stabilization of bread flavor is to isolate and identify the various compounds in bread which contribute to its flavor. Once the flavor components have been identified, research on the stabilization and enhancement of desirable bread flavor could be conducted more efficiently.

The flavor of bread has been discussed in several recent reviews (4,9,10,11), and intensive research on the problem is under way in a number of laboratories. Recently, Wiseblatt and Kohn (12), Fortmann (2), and Rotsch and Dörner (7) reported new compounds isolated and identified in their research on bread flavor.

The present paper describes the isolation and identification of several volatile carbonyl compounds occurring in fresh white bread. It presents an analytical approach different from those described in the recent literature, and expands the list of carbonyl compounds positively identified in bread so far.

Materials and Methods

Because white bread made by different formulations and/or analyzed by different procedures may give different analytical results, the

[1] Manuscript received February 23, 1960. Contribution from Western Regional Research Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Albany 10, California.

[2] Present address: Department of Chemistry, Montana State College, Bozeman, Montana.

materials and methods used in this work are described in detail.

Bread Extraction and 2,4-Dinitrophenylhydrazone Formation. The bread was made according to the following formulation:

	g
Unbleached baker's patent flour	1,000
Sugar	50
Yeast	30
Nonfat dry milk	30
Shortening	30
Salt	20
Yeast food	2.5
Water, 700 ml.	
Mixing time, 6 minutes	
Dough temperature, 27°C. (80°F.)	
Straight dough, 2 punches	
Fermentation time, 2 hours, 20 minutes, total	
Pan proof, 1 hour at 35°C. (95°F.)	
Baking time, 25 minutes at 218°C. (425°F.)	

Twenty-four 1-lb. loaves of the freshly-baked bread were immersed in 20 liters of carbonyl-free absolute ethanol while the loaves were still hot. After standing overnight at about 40° F., the bread was broken up into smaller pieces with a stirrer, and the alcoholic bread suspension was filtered through a large Büchner funnel.

The filtered alcoholic bread extract was passed through 1 by 40-in. Dowex 50[3] (H^+ form, 20–50 mesh) ion exchange column, then through a 1 by 40-in. Amberlite IR-4B (OH^- form) column. The extract was then distilled in 1½- to 2-liter batches at atmospheric pressure through a 14-in. Vigreux column, until the combined pot residue was about 1½ liters. The distillate was condensed into a 2,4-dinitrophenylhydrazine reagent (50 ml. of a saturated 80:20 ethanol-concentrated hydrochloric acid (v/v) solution for each 2 to 3 liters of distillate). This reaction mixture then was distilled at atmospheric pressure down to 1½ liters. It was further concentrated on a steam bath; most of the ethanol had now boiled away, leaving a precipitate of 2,4-dinitrophenylhydrazones (hereafter abbreviated 2,4-DNPH's) in excess 2,4-dinitrophenylhydrazine solution.

The 1½-liter portion of the bread extract remaining in the pot was further distilled, until the material in the pot would no longer distill over without becoming dark. The distillate was collected in a second portion of 2,4-dinitrophenylhydrazine reagent.

After overnight standing, the precipitated 2,4-DNPH's of each portion were filtered off separately, washed thoroughly, and dried

[3] Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

slowly at room temperature in air. Second crops of precipitated 2,4-DNPH's were filtered off after the filtrates had stood at room temperature for about a week. Before the 2,4-DNPH's were analyzed by chromatographic techniques, each second crop of 2,4-DNPH's was combined with its corresponding main crop.

Column Chromatographic Analyses. By the use of three-column chromatographic systems, five carbonyl compounds were isolated and identified. The three systems are described below.

The 2,4-DNPH samples from bread were chromatographed on a series of 2:1 silicic acid-Celite (w/w) columns according to the method of Pippen *et al.* (5), care being taken not to exceed the capacity of each column. Chloroform was the sample solvent, and diethyl ether-Skellysolve F mixtures, with the ether concentration progressively increasing, were the developing solvent mixtures. A large part of each of the 2,4-DNPH samples was insoluble in the sample solvent, chloroform. This chloroform-insoluble part, believed to be mostly bis-2,4-DNPH's of vicinal-dicarbonyl compounds, was not analyzed in the present work.

The second column chromatographic method used is an adaptation of a method by Ramsey and Patterson (6). Nitromethane (6 to 8 ml.) was ground into activated silicic acid (10 g.) with mortar and pestle. The silicic acid mixture was made into a slurry with Skellysolve B (in place of hexane), and the slurry was packed into a 9-mm.-diameter glass column.

The third method employed a 9-mm.-diameter column containing unactivated silicic acid (Mallinckrodt No. 2847 analytical reagent, 100 mesh), which was packed as a slurry with 3:7 benzene-Skellysolve B (v/v, all solvents redistilled) and developed with this solvent mixture under nitrogen pressure of 8 to 10 p.s.i.

Paper-Chromatographic Analyses. Three paper-chromatographic systems for analyzing 2,4-DNPH's were used. These systems, together with ultraviolet spectrum data, helped identify seven carbonyl compounds, of which some identities are more nearly certain than others. (Two of these were also identified through column chromatography.)

The three systems employed include those of Nonaka *et al.* (3); Sundt and Winter (8), but applied to 2,4-DNPH's on Whatman No. 1 paper, impregnated with 25% dimethylformamide in acetone; and Ellis *et al.* (1). For each of the systems ultraviolet spectrum data were obtained directly on the paper chromatograms after the manner of Nonaka *et al.* (3). For the last two systems the chromatograms were thoroughly air-dried before their ultraviolet spectra were determined.

2,4-Dinitrophenylhydrazones. Generally, 2,4-DNPH's of carbonyl

compounds isolated from bread were recrystallized from 95% ethanol whenever enough 2,4-DNPH was available. Authentic carbonyl 2,4-DNPH's were obtained according to Pippen *et al.* (5), prepared mostly from commercially available carbonyl compounds. Ethyl pyruvate was prepared by conventional esterification methods, in which pyruvic acid was refluxed with an excess of absolute ethanol and a trace of sulfuric acid.

Oven Vapor Condensate. Some preliminary work was done on oven vapors from baking white bread. The vapors were condensed into a trap containing a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid, and the resulting 2,4-DNPH's were analyzed by column and paper chromatography as described previously.

Results and Discussion

Approximately 0.19 g. of chloroform-soluble 2,4-DNPH's of carbonyl compounds isolated from bread was characterized. Not analyzable by the methods used in this study was approximately 0.74 g. of 2,4-DNPH's which are predominantly sparingly soluble in most common organic solvents. These sparingly soluble 2,4-DNPH's probably include biacetyl bis-2,4-DNPH, methylglyoxal bis-2,4-DNPH, and additional furfural 2,4-DNPH.

Five of the more than a dozen different 2,4-DNPH bands which separated on each chromatographic column were identified as follows after similar bands were combined:

Acetaldehyde was isolated from bread as its 2,4-DNPH (140 mg.), which melted at 158.5°–168°C. (Probably the derivative is a mixture of two crystalline forms of acetaldehyde 2,4-DNPH, or contains a trace of impurities.) The mixed melting point with authentic acetaldehyde 2,4-DNPH (m.p. 167.5°–169°C.) was 159°–168°C. The infrared spectra of the natural and authentic materials appear to be identical.

Ethyl pyruvate was identified by the fact that a mixed melting point of its 2,4-DNPH (40 mg., m.p. 158.5°–159°C.) with authentic ethyl pyruvate 2,4-DNPH (m.p. 157.3°–158°C.) was undepressed (157.3°–158°C.). The infrared spectra of the natural and synthetic materials are identical. It is not known whether the ethyl pyruvate existed in bread as such or as pyruvic acid which was esterified during the extraction.

Furfural was identified through its 2,4-DNPH (3 mg., m.p. 219°C.), which was recrystallized from hot pyridine plus water. When it was mixed with authentic furfural 2,4-DNPH (m.p. 227°C.), the mixed melting point was not depressed (m.p. 220.5°–221.5°C.). Infrared spectra are essentially identical.

Hexanal and 2-methylbutanal were identified as a result of the separation of their 2,4-DNPH's on a nitromethane-silicic acid column. The isolated hexanal 2,4-DNPH (2 mg., m.p. 109°–110°C.), when melted with authentic hexanal 2,4-DNPH (m.p. 109°C.), showed no melting point depression (m.p. 110.5°–111.5°C.). Not enough material was left for infrared analysis, but co-chromatography of the sample with hexanal 2,4-DNPH produced only one zone on a silicic acid column impregnated with nitromethane, and only one spot on a 2-phenoxyethanol-impregnated paper chromatogram.

2-Methylbutanal 2,4-DNPH (0.7 mg.) was identified by melting point (m.p. 122°–126°C.), by R_f and ultraviolet spectra obtained on 2-phenoxyethanol-impregnated paper, and by the observation that only one spot was formed when the sample was mixed with synthetic 2-methylbutanal 2,4-DNPH and chromatographed together on 2-phenoxyethanol-impregnated paper. Unfortunately the sample was depleted before a mixed melting point could be taken with authentic 2-methylbutanal 2,4-DNPH (m.p. 131°–131.2°C.).

The major carbonyl components in freshly baked white bread, not considering chloroform-insoluble derivatives, appear to be principally acetaldehyde (1 part), ethyl pyruvate (0.25 part), furfural (0.02 part, probably a low estimate, since furfural 2,4-DNPH borders on the chloroform-insoluble class of 2,4-DNPH's), and lesser amounts of hexanal and 2-methylbutanal. The quantities are not very meaningful, because the procedure used in the extraction of bread may not afford quantitative results.

Additional small quantities of 2,4-DNPH's, of generally less than 1 mg. each, constituted the remainder of the 2,4-DNPH bands from the chromatographic columns. The bands, when eluted, had indistinct melting points and were often oily solids. Several of the substances present in such small quantities were identified by use of a microanalytical technique involving paper chromatography coupled with ultraviolet spectrum analysis (3). It was felt that adequate proof of the identities of these minute samples was obtained if the R_f values and ultraviolet spectrum data of an unknown, obtained from at least three widely differing paper chromatographic systems, agreed closely with those of an authentic 2,4-DNPH.

Table I compares data of 2,4-DNPH's of carbonyl compounds isolated from bread with those of authentic 2,4-DNPH's. Bread carbonyl compounds identified by use of the microanalytical technique include acetone, formaldehyde, and methyl ethyl ketone, as well as the previously identified acetaldehyde and ethyl pyruvate. Interestingly, isobutyraldehyde and n-valeraldehyde were identified (together

with acetaldehyde and acetone) in the same manner in oven vapors from baking bread, but were not found in the ethanol extract of bread.

TABLE I

COMPARISON OF R_f VALUES AND ULTRAVIOLET SPECTRUM MAXIMA AND MINIMA OF 2,4-DINITROPHENYLHYDRAZONES OF ISOLATED BREAD CARBONYLS AND AUTHENTIC CARBONYLS

Compound Suspected	R_f of 2,4-DNPH		Absorption Maxima of 2,4-DNPH		Absorption Minima of 2,4-DNPH	
	Isolated	Authentic	Isolated	Authentic	Isolated	Authentic
			mμ	mμ	mμ	mμ
	2-Phenoxyethanol:Heptane System					
Formaldehyde	0.16	0.16	362	360–1	301	299–300
Acetaldehyde	0.20	0.20	368	368	300	301
Ethyl pyruvate	0.44	0.44	369	368	304	303
Acetone	0.49	0.49	372	372	305	302
Methyl ethyl ketone	0.73	0.74	374	374	308	308
Isobutyraldehyde[a]	0.63	0.63	369	368	305	301
n-Valeraldehyde[a]	0.70	0.70	368	368	305	301
	N, N-Dimethylformamide:Decalin System					
Formaldehyde	0.09	0.09	361–3	358–9	297	297–9
Acetaldehyde	0.17	0.16	366	365–6	301	300
Ethyl pyruvate	0.29	0.27	365–6	365–6	301	302
Acetone	0.27	0.27	368	368–9	302	301
Methyl ethyl ketone	0.49	0.51	369	368	308	307
Isobutyraldehyde[a]	0.38	0.39	364	364	302–4	299
n-Valeraldehyde[a]	0.43	0.44	365	364	299	299
	Propylene Glycol:Methanol:Skellysolve C System					
Formaldehyde	0.15	0.18	364	364	295	294
Acetaldehyde	0.39	0.39	372–3	372	305	306–7
Ethyl pyruvate	0.66	0.74	368	367–8	302	298
Acetone	0.68	0.65	373	372–3	302	297
Methyl ethyl ketone[b]	0.92	0.91	368	369	319	317
Isobutyraldehyde[a]	0.82	0.82	368–9	368	302–4	302–3
n-Valeraldehyde[a]	0.93	0.93	367–8	367	299	300–2

[a] Found in oven vapors from baking bread, but not in the ethanol extract of bread.
[b] Used 34% ethylene glycol in place of 20% propylene glycol, because of streaking with latter.

In the different paper chromatographic systems used, most of the trace samples of bread carbonyl 2,4-DNPH's resolved into many spots each. Most of these spots gave ultraviolet spectra which are incongruous with those of known 2,4-DNPH's at hand. Probably some are decomposition products, but many are probably still unresolved mixtures of 2,4-DNPH's. Others may yet be unknown 2,4-DNPH's. The seven compounds listed in Table I are the only ones whose spectrum data and R_f values in several systems agree fairly well with those of known 2,4-DNPH's. The known carbonyls whose 2,4-DNPH's

were used for comparisons included those mentioned previously as being found in bread (4,12).

The R_f values in Table I are not absolute; the R_f of a 2,4-DNPH occasionally varied from run to run. Thus it was necessary to run unknowns and suspected knowns side by side on the same chromatogram. However, the R_f value of one 2,4-DNPH relative to another remained in all cases fairly constant.

When used alone, the ultraviolet spectrum maxima and minima are not sufficiently definitive, since similar carbonyl compounds in general give similar maxima and minima. Thus, for any one chromatographic system, all normal aliphatic aldehyde 2,4-DNPH's (except the first member, formaldehyde 2,4-DNPH) have the same maximum and minimum.

Of the ten carbonyl compounds identified in this work, 2-methylbutanal has to our knowledge not yet been reported for bread. Acetaldehyde, ethyl pyruvate, furfural, hexanal, acetone, and formaldehyde were mentioned in recent literature (see 2,7,12); but methyl ethyl ketone, isobutyraldehyde, and n-valeraldehyde have been mentioned only in early literature and with evidence probably insufficient to establish their specific identity.

Acknowledgments

The authors are indebted to M. Hanamoto for assistance in the earlier part of this work, to M. Nonaka and E. L. Pippen for aid and for samples of some authentic carbonyl derivatives, and to E. Gong and G. F. Bailey for spectrophotometric analyses.

Literature Cited

1. Ellis, R., Gaddis, A. M., and Currie, G. T. Paper chromatography of 2,4-dinitrophenylhydrazones of saturated aliphatic aldehydes. Anal. Chem. **30:** 475–479 (1958).
2. Fortmann, K. L. Technology of continuous dough processing. Cereal Science Today **4:** 290–292, 308 (1959).
3. Nonaka, M., Pippen, E. L., and Bailey, G. F. Separation and identification of n-alkanals, 2-alkanones, 2-alkenals, and 2,4-alkadienals by paper chromatography and direct spectrophotometric examination. Anal. Chem. **31:** 875–877 (1959).
4. Otterbacher, T. J. A review of some technical aspects of bread flavor. Baker's Digest **33** (3): 36–38, 40, 42 (June 1959).
5. Pippen, E. L., Eyring, E. J., and Nonaka, M. Chromatographic separation of 2,4-dinitrophenylhydrazones. Anal. Chem. **29:** 1305–1307 (1957).
6. Ramsey, L. L., and Patterson, W. I. Separation and purification of some constituents of commercial hexachlorocyclohexane. J. Assoc. Offic. Agr. Chemists **29:** 337–346 (1946).
7. Rotsch, A., and Dörner, H. Neue experimentelle Erkenntnisse über das Backaroma. Brot u. Gebäck **11:** 173–177 (1957).
8. Sundt, E., and Winter, M. Paper chromatography of 3,5-dinitrobenzoates of alcohols. Anal. Chem. **29:** 851–852 (1957).
9. Sykes, H. G. The behavior of yeast in pre-ferments and bread doughs. Baker's Digest **33** (4): 48–51 (August 1959).

10. WISEBLATT, L. The flavor of bread. Northwestern Miller 258(11): 1a, 16a-17a (September 10, 1957); Bread flavor.
11. WISEBLATT, L. Chemistry of natural food flavors — A symposium. Quartermaster Food and Container Institute (1957), pp. 145–151.
12. WISEBLATT, L., and KOHN, F. E. Some volatile aromatic compounds in fresh bread. Cereal Chem. 37: 55–66 (1960).

PARTICLE SIZE IN AQUEOUS STARCH DISPERSIONS[1]

G. E. BABCOCK, R. TOBIN, R. J. DIMLER, AND F. R. SENTI

ABSTRACT

Particle size in starch dispersions prepared by commonly used methods or by high-shear treatments was examined by ultracentrifugal and light-scattering methods. Heating at 98° or 120°C. in water left nearly 70% of the corn starch as relatively large, incompletely dispersed granule fragments which had sedimentation coefficients S_{20} greater than 1,500S (Svedberg units). About one-half the dispersed starch was free amylose, which sedimented as a separate component with $S_{20} = 5–6S$. Remaining dispersed particles had sedimentation coefficients up to 600S. Most marked effect of sodium hydroxide (1.0*N*) on autoclaved solutions was to free all the amylose component with little change in the degree of dispersion of the amylopectin residue. Ammonia pregelatinization weakened the granule structure of corn starch and doubled the extent to which it was dispersed in water at 98°. High rate of mechanical shear was effective in dispersing granule fragments in starch pastes. Average particle size could be reduced readily to a narrow distribution with $S_{20} = 80–150S$ and particle weights of 80–150 million. Little difference was found in the extent of dispersion of corn and wheat starch pastes under shear. Amount of free amylose in solution was relatively unaffected by reduction in particle size and comprised about 60 to 75% of the total amylose content of the starch.

The swelling behavior of starch granules on heating in water is well known; but the extent of dispersion or disaggregation of the gelatinized starch has received relatively little study. The granules swell rapidly when the temperature of the water reaches about 60°C.; the extent of swelling generally increases with a further rise in temperature. When fully swollen or gelatinized by heating for a few minutes at 100°C., granules of corn starch increase manyfold in volume and appear as gelatinous particles. At least part of the starch

[1] Manuscript received January 29, 1960. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Parts of this paper were presented at the 127th meeting of the American Chemical Society, Cincinnati, Ohio, March 29-April 7, 1955.

is dispersed sufficiently so that particles or molecules can diffuse through the swollen gel particle and enter the solution.

Although the swollen granules of some starches spontaneously disintegrate on continued heating near 100°C., corn starch granules are relatively resistant and suffer little damage unless subjected to higher temperatures or to mechanical shearing forces. Complete rupture of granules occurs, for example, if a corn starch suspension is vigorously stirred while pasted at 100°C. Undispersed fragments of granules still remain in the starch solution and can be observed with an ordinary microscope. The use of a phase microscope enabled Bechtel (1) to detect particulate matter as well as remnants of granule fragments in starch solutions prepared by dispersion in alkali, by autoclaving at 125°C., or by treatment in a Waring Blendor[2] after pasting at 96°C. No estimates have been made of the proportion of such incompletely dispersed starch and no data have been obtained on the size of particles below the limit of resolution of the microscope.

An investigation was undertaken to obtain more information on the range of particle sizes present in corn starch dispersions prepared by conventional methods and to study particularly the effect of shearing forces on particle size. The analytical ultracentrifuge is well adapted for this purpose since, by adjusting the rotor speed, sedimentation over an extremely wide range of particle weights can be observed. The data on sedimentation coefficients of constituent particles was supplemented by light-scattering measurements on some of the starch dispersions to establish an approximate relationship between sedimentation coefficient and particle weight.

Experimental

Starch Samples. Commercial corn starch samples were obtained through the courtesy of the Corn Products Company, Argo, Illinois; A. E. Staley Manufacturing Company, Decatur, Illinois; and the Clinton Corn Processing Company, Clinton, Iowa. Laboratory-prepared corn starch was isolated from No. 1 Schwenk hybrid corn by wet-milling subsequent to water-steeping at 37°C. for 48 hours. Wheat starch was prepared in the laboratory by a wet-milling process after steeping of the grain at 37°C. Commercial wheat starch was supplied by the Huron Milling Division, Hercules Powder Co., Harbor Beach, Michigan.

Preparation of Starch Dispersions. Starch dispersions were prepared by pasting 1% suspensions for 0.5 to 2 hours at 98°C. with mechanical

[2] Mention of a commercial product or a trade name does not constitute an endorsement by the U. S. Department of Agriculture.

stirring. Solutions were stirred with a magnetic- or propeller-type stirrer at speeds under 250 r.p.m. Prolonged stirring at these low speeds did not affect the size distribution of the pasted dispersions.

Several additional treatments were given to starches after preliminary dispersion in water at 98°C. for 30 minutes. These treatments included autoclaving at 120°C. for 0.5 to 1.5 hours, stirring in a No. 700 Waring Blendor for 15 and 45 minutes, and shearing by forcing through a rayon-type spinnerette. Holes in the spinnerette were 0.003 in. in diameter. Rate of flow and shear gradient were controlled by regulating the pressure of the compressed nitrogen applied to the dispersion. Action of a Gaulin laboratory homogenizer on 1% pastes of gelatinized starch was also studied. In this apparatus the starch dispersion was forced through the small clearance between a valve and seat under high pressure. Pastes were passed through the homogenizer at pressures of 1,000, 2,000, and 3,000 p.s.i. All dispersions were diluted to 0.5% concentration before sedimentation measurements were made.

Laboratory-prepared corn starch was dispersed directly in 0.5*N* sodium hydroxide solution which had been sparged thoroughly with nitrogen. Aliquots of this solution were sparged with oxygen, and sedimentation measurements were made on both sets of solutions over a period of 30 days.

Sedimentation patterns of the starch dispersions were obtained with a Spinco Model E ultracentrifuge using a 30-mm. double-sector epon-resin centerpiece with the starch dispersion in one sector and the solvent in the other. The resulting baseline on each photograph facilitated area measurements. The centrifuge was operated first at 12,590 r.p.m. (11,500 × G), after which the speed was raised to 35,600 r.p.m. (96,200 × G). Three classes of starch particles were differentiated in this manner: (a) incompletely dispersed particles that reached the bottom of the cell by the time the first pattern was photographed, 8 minutes after the centrifuge reached full initial operating speed (estimated sedimentation coefficient 1,500 Svedberg units and larger); (b) rapidly sedimenting particles having sedimentation coefficients up to 600S (Svedberg units) and having particle weights in the multimillions (primarily amylopectin); and (c) slowly sedimenting material requiring the higher rotor speed and having sedimentation coefficient in the region of 5 to 6S (primarily amylose).

Sedimentation coefficients were measured by a method of Svedberg (9). Areas of patterns were calculated by a formula of Pickels (7) using 0.00151 as the specific refractive increment of starch in water dispersions and 0.00160 for alkali dispersions. The specific refractive increment of starch in alkali was determined from a sedimentation

pattern of known concentration of amylose. To facilitate comparison of samples, the sedimentation constant-concentration relationship of isolated amylose and amylopectin was determined, and sedimentation coefficients were adjusted to a common concentration. In most cases this correction was small.

Concentration of starch in the dispersions was determined by the anthrone method (8). The observed area under a peak of the sedimentation pattern divided by the area calculated as equivalent to the total concentration of starch in the starch dispersion gave the fraction of the carbohydrate observed in that peak. The dispersed starch was considered to be the sum of the rapidly sedimenting particles (amylopectin) and the slowly sedimenting particles (amylose). The remainder was considered to be the incompletely dispersed starch, the particles of which reached the bottom of the cell too quickly to be included in the pattern.

Results and Discussion

Starch Dispersed by Heating and Low-Speed Stirring. The sedimentation pattern represented by the solid line in Fig. 1 is the type

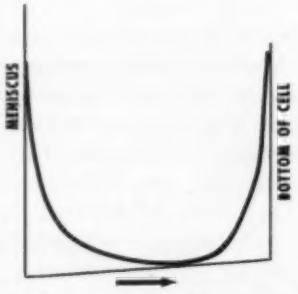


Fig. 1. Sedimentation pattern of corn starch dispersed by autoclaving (centrifuge rotor speed 12,590 r.p.m.).

obtained at the lower rotor speed (12,590 r.p.m.) when laboratory-prepared corn starch was dispersed by pasting at 98°C. for 30 minutes with low-speed mechanical stirring, followed by autoclaving at 120°C. for 3 hours. The left portion of Fig. 1 results from rapidly sedimenting particles which have not yet all moved away from the meniscus. These have a rather broad weight distribution as shown by the extension of the curve past the center of the cell. The sedimentation coefficients of the fast component range up to 600S. The fast-sedimenting particles comprise only about 13% of the total starch

in the dispersion with another 18% of the starch in a slow-sedimenting component which has not moved away appreciably from the meniscus in Fig. 1. The slow component has a sedimentation coefficient of 5 to 6S and was identified as amylose, on the basis of iodine sorption in separate experiments. The remaining 69% of the starch in the dispersion must be accounted for as incompletely dispersed material which sedimented to the bottom of the ultracentrifuge cell before the rotor attained full operating speed. This material gives rise to the concentration gradient at the extreme right of Fig. 1. These extremely large particles are referred to hereafter as incompletely dispersed starch and must include those granule fragments observed with the optical microscope as well as swollen gelatinous fragments which differ too little in refractive index from the solvent to be seen with the microscope.

The use of milder conditions (2 hours at 98°C. instead of 3 hours at 120°C.) made very little difference in the state of dispersion of the starch (Table I). The amount of incompletely dispersed starch was increased slightly (71% compared with 69%), the amount of fast component being unchanged at 13%, and that of the slow component (amylose) having dropped from 18 to 16% of the total starch. Pretreatment of the starch by gelatinization in liquid ammonia (5) prior to pasting at 98°C. doubled the amount of starch dispersed (Table I). The quantity of slow-sedimenting or amylose component increased

TABLE I

EFFECT OF PASTING CONDITIONS ON EXTENT OF DISPERSION OF CORN STARCH IN AQUEOUS SOLUTIONS

STARCH PRETREATMENT	DISPERSION CONDITIONS	DISPERSED STARCH [a]	
		Total	Amylose
		%	%
None	2 hours at 98°	29	16
None	3 hours at 120°	31	18
NH_3-gelatinized	2 hours at 98°	62	20
[b]	1*N* NaOH at 25°	41	29

[a] "Dispersed starch" is defined as the fraction that has sufficiently small particle size to sediment at an observable rate at 11,500 × *g* under conditions of operation of the ultracentrifuge.
[b] Pasted at 120° for 3 hours prior to addition of alkali.

relatively little (20 vs. 16%), the major change having occurred in the amount of fast-sedimenting component (42% instead of 13% of the total starch). Incompletely dispersed starch in this case was only 38% compared with 71% when untreated starch was used. The pretreatment with liquid ammonia improved greatly the dispersibility of the starch, yet nearly one-fourth of the amylose in the starch failed to be released as a slow-sedimenting component.

Dispersion in Alkali. An increased degree of dispersion was obtained when the starch was dispersed with 1*N* sodium hydroxide solution at 25°C. as compared with autoclaving in water alone (Table I). In contrast to pretreatment with liquid ammonia, alkali increased considerably the amount of slow-sedimenting, or amylose component, while leaving the amount of fast-sedimenting component relatively unchanged. This result indicates a preferential solubility of the amylose component in alkali; the amount liberated from the starch was essentially that predicted from iodine sorption measurements of amylose content of starch. Baum and Gilbert (3) recently used this preferential solubility of amylose in alkali as a basis for fractionating potato starch.

Since oxidative degradation of starch is considered to occur in the presence of alkali, a comparison was made between an alkaline starch dispersion exposed to oxygen and one exposed to nitrogen. Sedimentation patterns showed no significant difference between the portion of the alkaline dispersion sparged with nitrogen and that sparged with oxygen and allowed to stand at room temperature up to 30 days (Table II). In both cases the amount of slow-sedimenting

TABLE II

EFFECT OF 0.5*N* SODIUM HYDROXIDE ON DISPERSION OF CORN STARCH IN PRESENCE OF OXYGEN AND OF NITROGEN

Time Standing	Treatment	S_{20} Slow Component	Dispersed Starch	
			Slow Component	Total
days			%	%
1	Nitrogen	6.02	30	55
2	Oxygen	6.21	28	71
3	Nitrogen	6.42	28	72
7	Oxygen	5.50	29	87
13	Nitrogen	5.48	30	90
13	Oxygen	4.97	30	91
30	Nitrogen	4.46	40	93
30	Oxygen	4.81	39	94

component remained essentially constant at 30% of the total starch during the first 13 days of standing. The samples analyzed after 30 days showed an increase in amount of slow-sedimenting component to a value of 40% and a spread in the distribution of particle weights, probably as a result of breakdown of larger particles. A gradual decrease in particle size of the slow-sedimenting component is indicated by the lower sedimentation constants for samples which stood more than 3 days. The total amount of starch dispersed increased with time from 55% after 1 day to 93–94% after 30 days, indicating a pro-

gressive breakdown of the large granule fragments. The resulting rapidly sedimenting particles had too broad a distribution of particle size to permit reliable measurements; however, the average sedimentation coefficient at 0.5% concentration dropped from 100S after 1 day's standing to 35S after 30 days' standing, again indicating the occurrence of chemical degradation. However, this degradation appeared to be independent of the presence of large quantities of oxygen.

Dispersion of Pasted Starch by High-Speed Stirring. In contrast to the relatively incomplete disruption of swollen granule fragments in starch pasted at 98°C. by low-speed mechanical stirring, the shear developed in a high-speed laboratory blender effected a marked reduction in particle size of the gelatinized starch (Table III). The area under the amylose peak, together with that under the main peak

TABLE III

SEDIMENTATION COEFFICIENTS AND PARTICLE WEIGHTS OF STARCH DISPERSED IN A BLENDER AFTER GELATINIZATION AT 98°C.

STARCH SAMPLE	TREATMENT	DISPERSED STARCH			S_{20} FAST COMPONENT[a]	PARTICLE WEIGHT[b]
		Slow Component	Fast Component	Total		
	minutes	%	%	%		
Commercial corn B	15	17	66	83	106	115×10^6
Commercial corn B	45	18	75	93	80	80×10^6
Laboratory-prepared corn	15	20	67	87	110	145×10^6
Laboratory-prepared corn	45	20	70	90	91	....
Commercial wheat	15	19	70	89	112	....
Commercial wheat	45	19	70	89	84	....

[a] Corrected to $C = 0.3\%$.
[b] Measured by light-scattering method.

of the pattern, accounted for up to 93% of the starch in the dispersion. There appeared to be no marked difference in the dispersibility of the commercial starches compared to the laboratory-prepared corn starch. Extent of dispersion as well as the sedimentation coefficients of the fast-moving peak was about the same for the two types of starches. If commercial corn starch was kept in the blender 45 minutes instead of 15, the average particle size dropped from 115 million to 80 million. Despite this reduction in particle size, the free amylose in solution only increased from 17 to 18%. Since corn starch contains about 27% amylose, the remaining amylose must be firmly bound to the amylopectin in the fast-sedimenting particles.

Shearing by Forcing Pastes through Small Orifices. A more extensive study of the effect of shear on the particle size of corn starch

was carried out by forcing starch pastes through the capillary openings of a rayon-type spinnerette. Rate of shear was controlled by the pressure on the solution in the spinnerette, and the amount of shearing was varied by the number of times the solution was passed through the spinnerette.

Ammonia-pregelatinized starch was used to study the effect of shear on the dispersed starch because of the greater ease with which it is brought into essentially complete dispersion (30 passages through the spinnerette at only 10 p.s.i.). The sedimentation coefficient of the main component decreased to a limiting value, as shown in Table IV, on

TABLE IV
EFFECT OF SHEARING ON SEDIMENTATION COEFFICIENTS OF AMMONIA-PREGELATINIZED STARCH IN AQUEOUS DISPERSIONS

Shearing Conditions		S_{20} [b]
Passes [a]	Pressure	
	p.s.i.	
20	10	385
30	10	277
40	10	282
50	10	285
5	25	212
10	25	203
15	25	205
10	50	182
20	50	175
30	50	162
40	50	165

[a] Number of times through 0.003-in. spinnerette.
[b] Measured at $C = 0.3\%$.

repeated passage of the paste through the spinnerette at a given pressure. This limiting value varied with the pressure or rate of shear, being lower at higher rates of shear. The number of passes of the solution through the spinnerette required to reach the constant sedimentation coefficient also varied with the pressure and ranged from about 10 to 30 passes.

Untreated corn starches were much more difficult to disperse than was ammonia-pregelatinized starch. Thus, untreated starch after pasting for 30 minutes at 98°C. was dispersed to an average of only 80% of theoretical after 60 passages through the spinnerette at 50 p.s.i., compared with essentially complete dispersion of ammonia-pregelatinized starch by 30 passages at only 10 p.s.i. The changes in the sedimentation pattern of the fast sedimenting component from untreated starch on repeated passage of the dispersion through the spinnerette are shown in Fig 2. A marked reduction in the breadth of the distribution of particle sizes occurs, as evidenced by the decreasing width

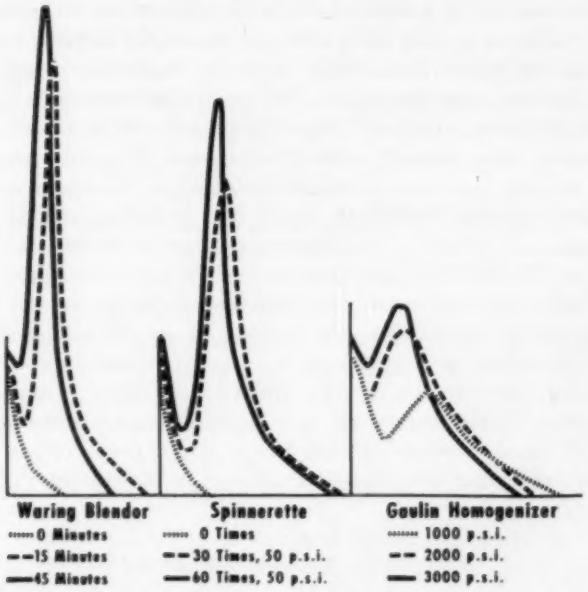


Fig. 2. Comparison of sedimentation patterns of starch pastes after shear treatment in a Waring Blendor, rayon-type spinnerette, and a Gaulin homogenizer. Patterns taken after 24 minutes at 12,950 r.p.m., 40° bar angle, C = 0.5%. Comparable to Fig. 1 but after a longer period of sedimentation and omitting the region at the bottom of the cell.

of the peak in the sedimentation patterns. A similar sharpening of the fast-sedimenting peak also occurred in starch dispersions subjected to the action of a Waring Blendor and, to a lesser degree, with a Gaulin homogenizer (Fig. 2).

High-shear gradients, developed in high-speed stirring or by passing starch pastes through narrow orifices, not only effectively reduce the particle size of the granule fragments but also reduce the breadth of the particle size distribution. Similar results have been reported for other high-polymeric substances when subjected to high-shear gradients (2). Particle size reduction was attributed to breakage of primary bonds and this probably occurs to some extent with the starch. Computation of the shearing stress in the spinnerette orifice gives a value comparable to one Bestul and Belcher (2) found sufficient to break primary bonds in high-molecular-weight polyisobutylenes. The results do not exclude, however, disaggregation through breakage of secondary bonds. In most of the cases listed in Tables III, IV, and V, the particle weight as given by the light-scattering

determination, or, as indicated by the sedimentation coefficients, was about equal to or greater than that determined for isolated amylopectin. Thus Stacy and Foster (10) report a molecular weight of 80 million for corn amylopectin in 1*N* potassium hydroxide solution, and Erlander and French (4) recently found a value of 168 million for immature dent and 45 million for mature dent amylopectin. Although the large particles in solution after shear treatment contained about 10% amylose, this factor would not contribute significantly to their molecular weight; it is apparent that most of the reduction in particle size could have come from secondary bond breakage.

Although the shear forces were sufficient to disaggregate or degrade the amylopectin appreciably, the amount of amylose in aqueous dispersion appearing in a sedimentation pattern never approached the approximate 28% in the original corn starch. Little if any increase of amylose occurred after shear treatment. The same incomplete liberation of amylose occurred with wheat starch preparations, despite their greater extent of dispersion after shear treatment (Table V).

TABLE V

EFFECT OF SPINNERETTE TREATMENT[a] ON PASTED STARCHES

STARCH SAMPLE	DISPERSED STARCH			S_{20}[b]
	Fast Component	Slow Component	Total	Fast Component
	%	%	%	
Commercial corn A	21	61	82	120
Commercial corn B	20	57	77	138
Commercial corn C	19	63	82	154
Laboratory-prepared corn	16	63	79	149
Laboratory-prepared wheat	19	77	96	156
Commercial wheat	19	71	90	139

[a] Pasted for 30 minutes at 98°C. with slow-mechanical stirring followed by 60 passes at 50 p. s. i. through a spinnerette having 0.003-in.-diameter holes.
[b] Corrected to $C = 0.3\%$.

About 25 to 40% of the amylose in both corn starch and wheat starch appears to remain attached to the amylopectin in aqueous dispersion after pasting and shear degradation by any of the three methods used—capillary spinnerette, high-speed propeller, or colloid mill. In contrast, aqueous alkali appears to liberate amylose completely from corn starch (Table I).

To confirm the identity of the slow-moving component as amylose and to check the analytical ultracentrifugal analysis of the concentration of free amylose, solutions were analyzed for amylose by the spectrophotometric iodine color method (6) after removal of the large par-

ticle components by centrifugation. After spinnerette treatment the dispersions were centrifuged at 105,000 × G for 15 minutes, a time calculated as sufficient to sediment most of the large particle components to the bottom of the tube and leave amylose in solution. Results show (Table VI) that the concentration of free amylose

TABLE VI

EFFECT OF SHEAR ON FREE AMYLOSE IN STARCH DISPERSIONS AS DETERMINED BY IODINE COLOR OF CENTRIFUGAL SUPERNATANTS

	Passes through Spinnerette[a]	Free Amylose, Percent	
		Iodine Color	Centrifuge Pattern
Granular Corn Starch	0	13.0	16.0
	5	16.4	15.8
	15	16.0	16.3
Ammonia-Pregelatinized Corn Starch	0	22.5	20.0
	1	23.5	20.9
	10	21.1	20.7

[a] Number of times starch paste was forced through 0.003-in. spinnerette at 25 p. s. i.

determined colorimetrically is in good agreement with that determined from the sedimentation patterns. Waxy maize starch dispersed at 97°C. failed to show a comparable slow-sedimenting component, thereby further substantiating identification of this component as amylose.

Literature Cited

1. BECHTEL, W. G. Examination of starch dispersions with the phase microscope. Cereal Chem. **28**: 29–39 (1951).
2. BESTUL, A. B., and BELCHER, H. V. Degradation of polyisobutylenes on shearing in solution. J. Appl. Phys. **24**: 1011–1014 (1953).
3. BAUM, H., and GILBERT, G. A. The fractionation of potato starch by centrifugation in alkali. J. Colloid Sci. **11**: 428–434 (1956).
4. ERLANDER, S. R., and FRENCH, D. Dispersion of starch granules and the validity of light-scattering results on amylopectin. J. Am. Chem. Soc. **80**: 4413–4420 (1958).
5. HODGE, J. E., KARJALA, S. A., and HILBERT, G. E. Methylation and ethylation of corn starch, amylose, and amylopectin in liquid ammonia. J. Am. Chem. Soc. **73**: 3312–3316 (1951).
6. KERR, R. W., and TRUBELL, O. R. Spectrophotometric analyses of starches. Paper Trade J. **117** (15): 25–28 (1943).
7. PICKELS, E. G. Methods of medical research, vol. 5, p. 121 (1952).
8. SCOTT, T. A., JR., and MELVIN, E. H. Determination of dextran with anthrone. Anal. Chem. **25**: 1656–1661 (1953).
9. SVEDBERG, T., and PEDERSON, K. O. The ultracentrifuge. Clarendon Press: Oxford (1940).
10. STACY, C. J., and FOSTER, J. F. Molecular weight heterogeneity in starch amylopectins. I. Weight and number average molecular weight. J. Polymer. Sci. **25**: 39–50 (1957).

PREPARATION AND PROPERTIES OF HYDROXYETHYLATED HIGH-AMYLOSE CORN STARCH[1]

J. C. RANKIN, J. G. RALL, C. R. RUSSELL, AND F. R. SENTI

ABSTRACT

Corn starch with an amylose content of 50% was hydroxyethylated in the dry state with ethylene oxide and an alkali catalyst to give products containing from 1 to 27% by weight of combined ethylene oxide in quantitative yields. Aqueous slurries of hydroxyethylated high-amylose starch (etherified to an extent of 5% or more) when cooked to temperatures between 73° and 90°C. gave pastes which were fluid in character and had improved dispersion stability on cooling and aging over that of the unmodified starch. In the cooking-cooling cycle the viscosity curves exhibited by these products are considerably lower than those of similarly substituted waxy and normal corn starches. Pastes of modified 50%-amylose starch are less translucent than those of like derivatives of the other two starches. Pasting characteristics of hydroxyethylated high-amylose starch are correlated with such variables of preparation as degree of substitution, alkali catalyst concentration, and pH of product. Microscopic examination and film-forming properties of their pastes are discussed.

Improved pasting properties obtained by hydroxyethylation of high-amylose starch indicate promise for this derivative of the new starch in industrial applications.

Resistance of the high-amylose starch granule to pasting in aqueous media at 92°C. or even higher temperature led Sloan, MacMasters, and Senti (16) to prewash corn starch granules containing 50% amylose (3,18) with mild alkali. Even then, temperatures between 140° and 170°C. are still needed to obtain a water dispersion of the pretreated starch, in contrast to the much lower cooking temperatures common in commercial practice to prepare starch or modified starch dispersions (4). The increasing availability and many projected uses of high-amylose starch (1,2,15) necessitated an examination of economical chemical modifications designed to render the new starch more readily dispersible in water. Kesler and Hjermstad (7,8,9) have improved solution stability of the amylose portion of normal corn starch by hydroxyethylation. Rankin, Mehltretter, and Senti have enhanced pasting properties of cereal flours by this reaction (12,13). High-amylose starch, therefore, was hydroxyethylated to obtain more readily dispersible products with improved paste stabilities. Other corn starches containing less amylose (normal 25% and waxy 0%

[1] Manuscript received November 5, 1959. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Presented at the 44th annual meeting, Washington, D.C., May 1959.

(5)) were similarly substituted for comparative purposes.

Evaluation studies on the modified starches included paste viscosity and clarity, microscopic observations, and film-forming properties. Correlated with these paste characteristics were such variables of preparation as degree of substitution, alkali catalyst concentration, and pH of product.

Preparation of Hydroxyethylated Corn Starches

High-amylose and waxy starches wet-milled from samples of Bear's Amicorn[2] and Iowa State College's Iowax 2, respectively, and normal commercial starch in dry mixtures with alkali catalyst were reacted with gaseous ethylene oxide under the conditions previously reported for cereal flours (12,13).

Batches (300 g. dry basis) of starch of approximately 14% moisture content were mixed thoroughly with dry, powdered sodium hydroxide and aged at room temperature for 3 days. Alkali-starches were then placed in a 1-qt. laboratory sigma-blade kneading machine. The kneading chamber was jacketed for circulation of temperature-controlling media. Attached to the cover of the mixing vessel was a combination vacuum-pressure gage to provide a crude estimation of reacted ethylene oxide. After air was evacuated from the vessel, gaseous ethylene oxide was introduced until atmospheric pressure was reached. As the mixture was kneaded the temperature was kept between 30° and 45°C., and the pressure dropped, owing to reaction of the ethylene oxide with the starch. At appropriate intervals as determined by gage readings, mixing was interrupted long enough to permit introduction of additional quantities of ethylene oxide. This operation was repeated as often as needed to supply sufficient ethylene oxide for the reaction to be completed and also to maintain a favorable reaction rate. Upon completion of runs the products were obtained in quantitative yields and in good granular condition, much like the original starch in appearance. The amount of ethylene oxide that had reacted was determined accurately by analyzing the sample for hydroxyethyl content by the method of Lortz (10). All ethylene oxide values reported in this paper were determined by analysis and were calculated as percentages of ethylene oxide in the final product on a dry, ash-free basis. An atmosphere of nitrogen instead of ethylene oxide was used for the control runs (starch and alkali).

The alkali catalyst was employed in concentrations of 0.25, 1.25, and 2.5%. Alkalinity of the modified starch in some experiments was

[2] Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

conveniently reduced by dry-blending with powdered acids or neutralizing gases as previously described (12,13). A number of neutralized products were washed or dialyzed with water to remove salt. This provided samples for determining effects of residual salt on pasting properties of hydroxyethylated starches. Losses incurred because of solubilization of products during removal of salt reduced quantitative yields to values between 89 and 71% for etherified high-amylose starches with ethylene oxide contents from 3 to 27%, respectively.

Ethylene oxide and other compressed gases used were commercial products. The acids and alkali used were of reagent grade.

Evaluation of Hydroxyethylated Corn Starches

It is well known that the ability of commercial starches to hydrate, disperse or dissolve, and to form films from the cooked pastes makes them highly adaptable for many industrial processes. Unlike waxy and normal starches, unmodified 50%-amylose starch does not have this ability under ordinary cooking procedures. Cooked slurries of the latter starch cast and dried on Plexiglas plates formed grainlike particles which were not held together in a continuous film. When unmodified high-amylose starch is dry-blended with proper amounts of alkali catalyst and then reacted with sufficient proportions of ethylene oxide, products do have the desired pasting properties. Table I illus-

TABLE I

EFFECT OF PREPARATIVE CONDITIONS ON CERTAIN DISPERSION CHARACTERISTICS OF HYDROXYETHYLATED HIGH-AMYLOSE CORN STARCHES

Reaction Conditions				Properties of Cooked Slurries		
Catalyst [a]	Temperature	Time	C_2H_4O	Viscosity and Film-Forming	Color [b]	Stability
%	°C	*hours*	%			
0.25	34	2	1	No	No	Settles
	43	5	5	Yes	No	Fluid
	43	11	7	Yes	No	Fluid
1.25	32	2	1	No	Yes	Settles
	45	3	5	Yes	Yes	Fluid
	..	..	5 [c]	Yes	No	Fluid
	45	7	7	Yes	Yes	Fluid
	..	..	7 [c]	Yes	No	Fluid
2.50	32	8	0	Yes	Yes	Gels
	30	2	3	Yes	Yes	Gels
	..	..	3 [c]	No	No	Settles
	33	5	7	Yes	Yes	Fluid
	..	..	7 [c]	Yes	No	Fluid
	33	13	12	Yes	No	Fluid

[a] Percentage based on combined weight of starch and NaOH.
[b] Yellow to orange.
[c] Neutralized products, pH 7.0.

trates conditions for obtaining hydroxyethylated high-amylose starches whose cooked slurries form pastes which have measurable viscosity, no color, and improved dispersion stability on aging. A yellow-to-orange color in some of the cooked pastes occurred when the residual alkali catalyst concentration was greater than 0.3%. This color may be attributed partially, at least, to the effect of alkali on protein at elevated temperatures. It has been found (15) that protein and starch in high-amylose corn are somewhat more difficult to separate from each other than they are in waxy and normal corn. The high-amylose starch contained 0.7% protein, which is considerably more than the 0.3% usually found in the two others. The alkali effect also prevailed, but to a lesser extent, in pastes of the other modified starches examined. Color formation can be prevented by either reacting the starch with ample ethylene oxide or neutralizing the product.

Viscosity. The most important single characteristic in industrial utilization of starch products is its viscosity—probably the most difficult property to evaluate. Workers have directed more attention to this determination than any other, but as yet no single method or group of methods is accepted as standard. In this study two different viscometers widely used by industry were employed. The Corn Industries viscometer (6) was employed as cited (13), and a Brabender Amylograph, such as that used extensively for starch viscosity measurements by Mazurs, Schoch, and Kite (11), was operated under different cooking conditions. Instead of adding starch slurries to a preheated viscometer, as was the case with the Corn Industries method of analysis, mixtures were heated in the amylograph from 25° up to 90°C.

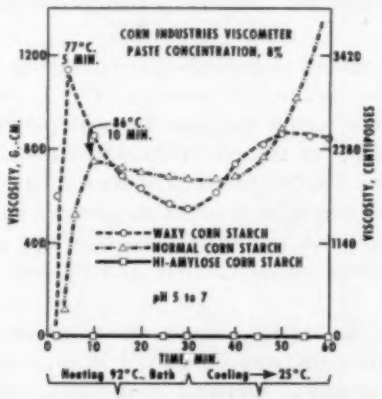


Fig. 1. Paste viscosity of unmodified corn starches.

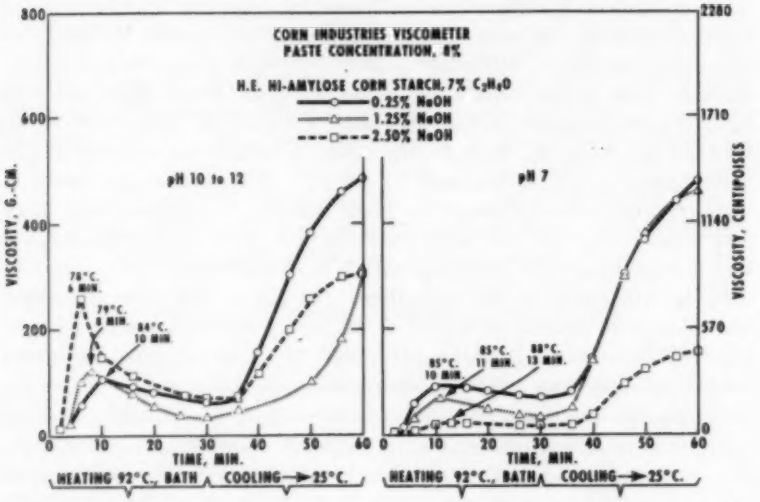


Fig. 2. Effects of catalyst content on paste viscosity of hydroxyethylated high-amylose starch (left) pH 10–12, and (right) of the same starch that had been neutralized, pH 7.

(1.5°C. per minute), held at 90°C. for 17 minutes, and then cooled at the same rate to 25°C. The bowl speed of the amylograph was 75 r.p.m. The complete cooking and cooling curves were plotted from viscosity charts of each instrument. Temperature at maximum hot viscosity is recorded on each curve. Two other significant points on these curves are also considered in this paper: minimum hot viscosity at 90°C. (for the heating time used) and maximum cold paste viscosity.

Although unmodified high-amylose starch under the cooking conditions used has no measurable viscosity (Fig. 1), after 7% hydroxyethylation the products gave the paste viscosity patterns shown in Fig. 2. The pasting curves of the etherified starch also depend upon the percentage of sodium hydroxide catalyst used in its preparation. This difference is further exemplified where the greatest change (lowering of both hot- and cold-paste viscosities) occurs in the product prepared with the highest amount of catalyst (2.5%) and neutralized with hydrochloric acid.

Comparison of the amylose content of corn starch as it affects viscosity of the hydroxyethylated product is illustrated by Fig. 3. Similarly substituted products prepared with 2.5% catalyst show 50%-amylose starch to be considerably lower in paste viscosity than normal

and waxy starches. These differ by approximately threefold and fivefold, respectively, for most of the points (mentioned above) of their pasting curves. The differences are further increased on neutralization

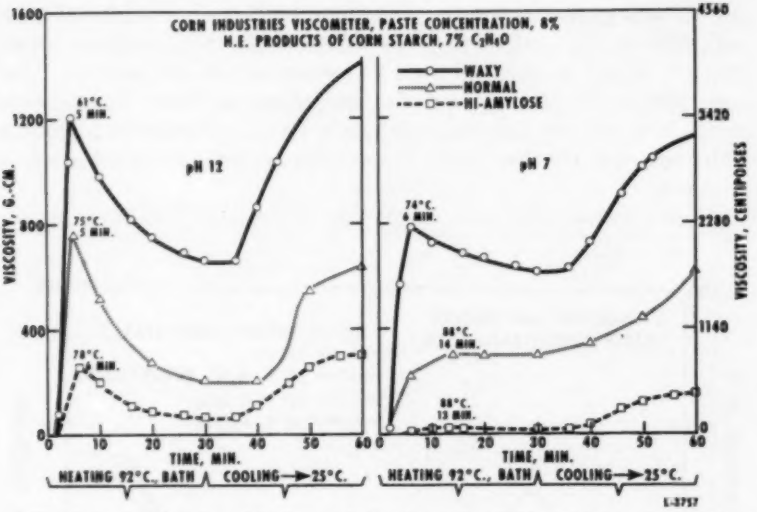


Fig. 3. Paste viscosity of hydroxyethylated starch, (left) pH 12, and (right) of the same starch that had been neutralized, pH 7.

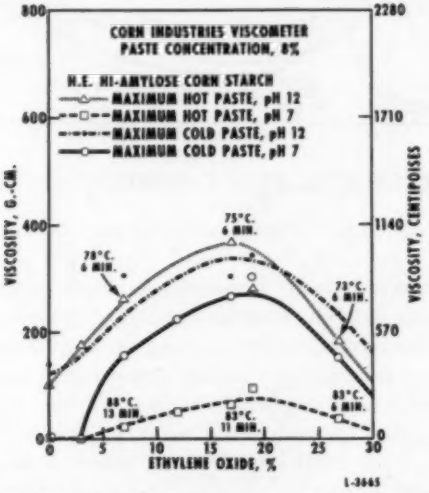


Fig. 4. Effect of ethylene oxide content on maximum hot and cold paste viscosity of hydroxyethylated high-amylose starch.

of the products with hydrochloric acid.

The influence of ethylene oxide content on maximum hot and cold viscosities of hydroxyethylated high-amylose starch is illustrated in Fig. 4. Progressively higher paste viscosities are indicated by these points from a control sample, containing 2.5% sodium hydroxide but not hydroxyethylated, through products with 19% ethylene oxide content. Values decrease above this percentage of modification. The same effects are observed for the neutralized product (hydrochloric acid); however, the latter samples have lower comparative viscosities which do not develop until a moderate degree of substitution is attained.

Figure 5 shows the effect of pH over a range of 4–12 on paste vis-

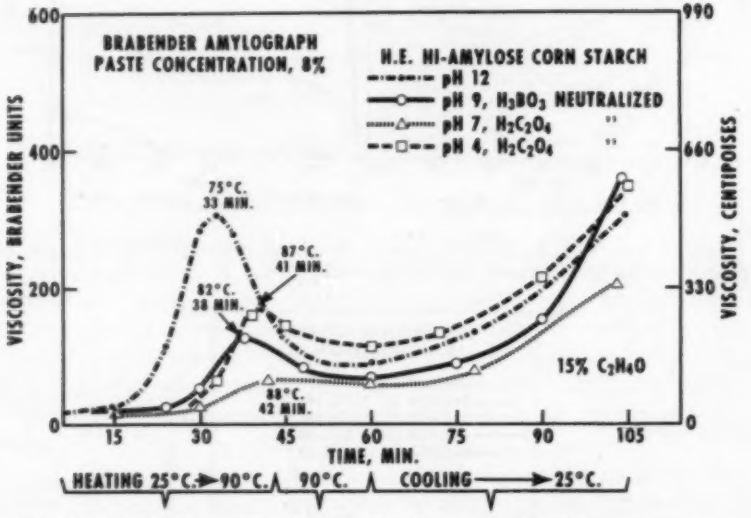


Fig. 5. Effect of pH on paste viscosity of hydroxyethylated high-amylose starch.

cosity of a hydroxyethylated high-amylose starch prepared with 2.5% sodium hydroxide catalyst. Practically all large differences seen in the pasting curves are located in the cooking cycle. During cooling of the pastes the rate of increase in viscosity was greatest for the boric acid-neutralized product, as expected, because of the well-known effect that borate complex formation has on starch paste viscosities. Maximum difference in over-all viscosity was observed between the products of pH 12 and 7.

Cooked 8% pastes of hydroxyethylated high-amylose starch stored

1 week showed no significant increases in viscosity ("set-back") at 25°C., as determined by Brookfield viscosity measurements.

Clarity. Paste clarity is another important index of the utility of modified starches, as the homogeneity of their cast films is dependent on the degree of paste dispersion, which is readily ascertained by clarity measurements. Determinations were carried out under previously reported cooking and measuring conditions (13). In some instances the aqueous slurry was neutralized with hydrochloric acid before cooking. As seen from Table II, greater changes in the paste clarity of a

TABLE II

PASTE CLARITY OF 7 PERCENT HYDROXYETHYLATED CORN STARCHES

SAMPLE	NaOH CATALYST	pH OF PRODUCT	PASTE CLARITY [a]		
			1% Paste	5% Paste	8% Paste
	%				
High-amylose	0.25	10	62	29	8
		7	64	31	7
	1.25	12	80	48	30
		7	79	45	14
	2.50	12	65	30	21
		7	29	5	6
Normal	2.50	12	89	78	38
		7	69	16	8
Waxy	2.50	12	91	89	84
		7	80	76	54

[a] Percentage transmission of light at 650 mμ measured with a spectrophotometer; 24-hour values based on distilled water as the standard for 100% transmission.

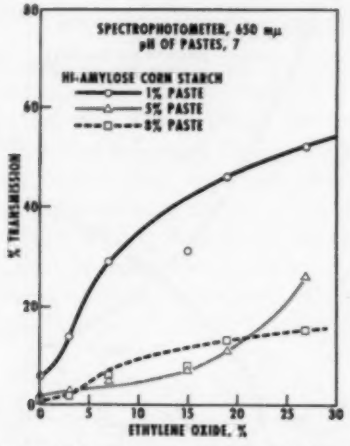


Fig. 6. Effect of ethylene oxide content on paste clarity of neutralized hydroxyethylated high-amylose starch.

7% hydroxyethylated high-amylose starch are effected by a variation in the pH of the tested products when 2.5% sodium hydroxide was employed for the etherification, as compared with the use of lower catalyst concentrations. In most cases, pastes did not attain the clarity of those of similarly substituted waxy and normal starches, regardless of the levels of catalyst and pH employed.

Clarity is also influenced by the ethylene oxide content, as shown in Fig. 6 for neutralized products of high-amylose starch. A gradation of the paste clarity values is observed from a control sample, containing the 2.5% sodium hydroxide catalyst but not hydroxyethylated, through products of 27% modification.

The results of clarity determinations made after 24 hours were not affected by allowing pastes to remain at room temperature for another 48 hours.

Influences of Paste Concentration and Storage of Products on Viscosity and Clarity. The effect of paste concentration of hydroxyethylated high-amylose starch on viscosity and clarity is illustrated in Fig. 7. In a product containing 19% ethylene oxide, maximum clarity is coincident with maximum hot and cold viscosities at a paste concentration of 12%. Etherified high-amylose starch with 2.5% sodium hydroxide catalyst shows only slight differences between its maximum hot and cold paste viscosities, neither of which increases as rapidly

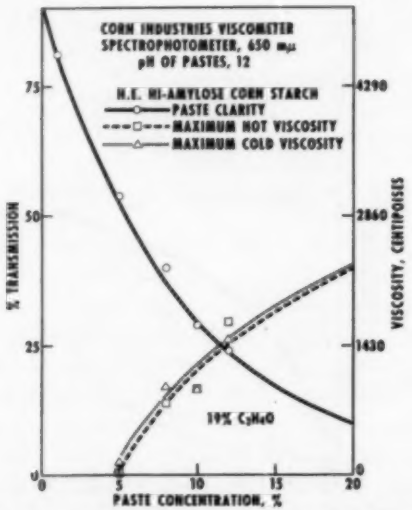


Fig. 7. Effect of concentration on paste viscosity and clarity of hydroxyethylated high-amylose starch.

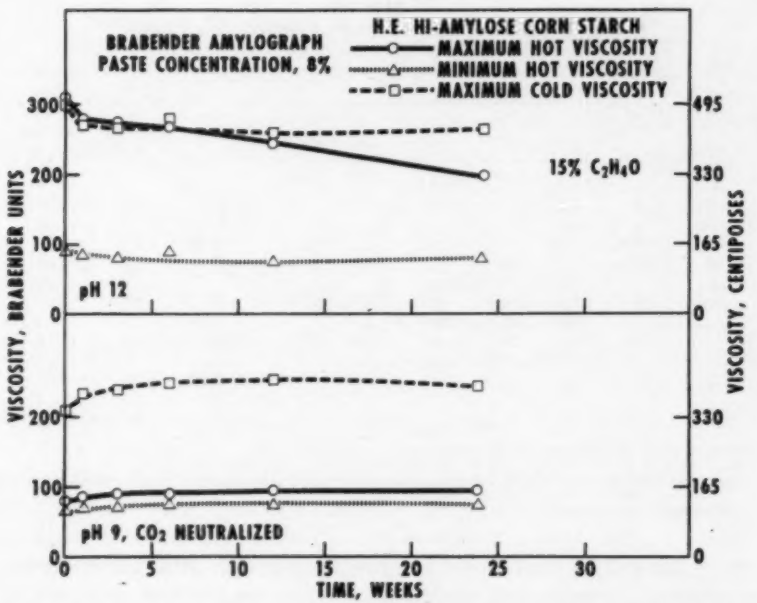


Fig. 8. Effect of aging of hydroxyethylated high-amylose starch on paste viscosity.

with increasing paste concentration as was observed with comparable derivatives of waxy and normal corn starches.

Data on stored hydroxyethylated products of high-amylose starch prepared with 2.5% sodium hydroxide catalyst are represented in Fig. 8. Samples with their normal moisture content were kept for 6 months at room temperature. The only major change in properties observed over this period was a lowering of the maximum hot-paste viscosity in the unneutralized sample (pH 12). Granular changes rather than molecular degradation are undoubtedly responsible for this lowering, as no significant decrease in either the hot minimum or cold maximum viscosities was observed. Paste clarities of the test products at 1, 5, and 8% concentration remained constant throughout the entire storage period. A more extensive study of effects of aging of hydroxyalkylated derivatives is now in progress and will be reported later.

Influence of Salt on Paste Viscosity and Clarity of Products. Table III shows paste evaluation data at three levels of hydroxyethylation of 50%-amylose starch. Alkali catalyst concentration of 2.5% was used in the etherifications. Comparisons are made of paste viscosity and

TABLE III

EFFECT OF RESIDUAL SALT ON PASTE VISCOSITY AND CLARITY OF HYDROXYETHYLATED HIGH-AMYLOSE CORN STARCHES[a]

C_2H_4O	pH of Product	Paste Viscosity[b]			Paste Clarity[c]		
		Hot Maximum	Cold		1% Paste	5% Paste	8% Paste
			55°C.	25°C.			
%		g-cm	g-cm	g-cm			
7	12	...	...	...	65	30	21
	7	...	...	...	29	5	6
	7[d]	...	...	...	43	11	7
12	12	259 (78°C., 6 minutes)	299	303	74	44	30
	7	49 (85°C., 10 minutes)	207	221	31	7	8
	7[d]	251 (81°C., 7 minutes)	347	371	58	22	14
27	12	...	...	...	79	50	39
	7	...	...	...	52	26	15
	7[d]	...	...	...	79	42	28

[a] Products prepared using 2.5% NaOH catalyst.
[b] Paste viscosity in 8% concentration was measured in the Corn Industries viscometer.
[c] Percentage transmission of light at 650 mμ measured with a spectrophotometer; 24-hour values based on distilled water as the standard for 100% transmission.
[d] Salt removed from samples.

clarity values obtained on the original hydroxyethylated starch, on the neutralized sample, and on the product when the resulting salt is removed. As would be expected, hot maximum viscosity was lowered considerably and the time and temperature required for its attainment were increased slightly by the presence of salts. Paste clarity was affected more by salts than were cold paste viscosities. Since yields were reduced on the average by about 16% in removing salts, a significant portion of the crude product representing the more soluble carbohydrates obviously was also removed. Therefore, all of the differences observed between properties of the crude and purified products, particularly in cold paste viscosities, should not be ascribed to the removal of salts. Similar effects were noted for pasting properties of like derivatives of waxy and normal starches. These data are not shown, as they do not change the comparative picture already presented for the three starches.

Microscopic Examination. In determining the character of, and changes in, starch and modified starch granules, a polarizing microscope with a magnification of 270 was used. Granule counts in 1% pastes and slurries were determined with the aid of a hemacytometer (14). The cooked pastes were prepared as noted for clarity tests and the slurries were made at room temperature.

Results of microexamination techniques are presented in Table IV. Slurries of unmodified and hydroxyethylated high-amylose starch con-

TABLE IV

MICROSCOPIC EXAMINATION OF HYDROXYETHYLATED CORN STARCHES

SAMPLE	NaOH CATALYST	C_2H_4O	pH OF PRODUCT	No. OF GRANULES[a] IN 0.1 MM.[3]	
				1% Slurry	1% Paste
	%	%			
High-amylose	...	..	7	4300	2200
	0.25	7	10	2900	1200
	1.25	7	12	2600	900
	2.50	7	12	2300	720
	2.50	7	7	2700	910
	2.50	15	12	1800	270
	2.50	27	12	1700	50
Normal	...	..	5	820	260
	2.50	7	12	650	20
Waxy	...	..	7	710	130
	2.50	7	12	890	1

[a] The number of granules observed in a hemacytometer.

tained considerably more and smaller granules than similar samples of waxy and normal corn starches per unit volume. The number of granules in slurries was progressively decreased with increased amounts of ethylene oxide, and the intensity of birefringence of remaining granules was likewise reduced. Their cooked pastes, as one would anticipate, contained a lower number of granules which continually decreased with increased substitution of ethylene oxide. Granules that remained undisintegrated in the paste exhibited no birefringence and were progressively swollen as the extent of modification increased. Granules were less affected by variations in catalyst concentration and pH of the product than they were by the degree of etherification.

Film. Present studies evaluate, in a comparative fashion, films of dried pastes obtained under the preceding cooking conditions. Cooked aqueous 5% dispersions of hydroxyethylated starches were cast on Plexiglas plates at 30°C. and were allowed to dry to an average thick-

TABLE V

FILM PROPERTIES OF HYDROXYETHYLATED CORN STARCHES

SAMPLE	NaOH CATALYST	C_2H_4O	pH OF PRODUCT	TENSILE STRENGTH	ELONGATION	DOUBLE FOLDS
	%	%		*lb/sq in*	%	
High-amylose	0.25	7	10	6000	6	14
	2.50	7	12	4000	3	3
	2.50	12	7[a]	4000	5	14
Normal	2.50	7	12	3000	[b]	[c]
	2.50	12	7[a]	1000	[b]	[c]
Waxy	2.50	7	12	3000	2	[c]

[a] Alkali removed from sample by dialysis.
[b] Values too small to measure.
[c] Breaks on insertion in testing machine.

ness of 0.033 mm. in a constant-humidity room. After equilibration at 50% relative humidity films were tested (17) for tensile strength, ultimate elongation, and folding endurance.

These results, compared in Table V, show that films prepared from derivatives of high-amylose starch have improved mechanical properties over those made from similar products of waxy and normal starches.

A visual observation of films cast from the hydroxyethylated products, as expected from paste clarity data (Table II), showed waxy to be the clearest, followed by normal and then high-amylose starches.

The ability to obtain films of these products with better mechanical properties would lie in the art and technique involved in preparing and casting solutions of the polymers; however, it is doubtful that the comparative positions of the hydroxyethylated starches would be changed.

Summary and Conclusions

Introduction of the hydroxyethyl group into starch may involve all hydroxyl groups of its repeating glucose units. It is theoretically possible to react every group and also possible to continue the reaction infinitely at each point of addition. Only a very low degree of substitution (1%) of the ether is needed to effect pronounced changes in the paste behavior of normal corn starch (7,8,9). The present study shows, however, that a moderate amount of ethylene oxide (5 to 7%) is required to render high-amylose starch amenable to a hot aqueous dispersion and to reduce its tendency to settle out. This ethylene oxide requirement is somewhat higher than that needed to impart comparable properties to normal starch and is, no doubt, mainly attributable to the higher amylose content of the new starch. Small differences noted in the molecular structure of amylopectin from high-amylose starch as compared with that from normal starch (15) may also be a contributing factor.

Sufficient hydroxyethylation of high-amylose starch (5% or above) imparts viscosity to cooked slurries of this starch derivative, and its viscosity may be varied by changing the degree of substitution of ethylene oxide. Hot and cold viscosities of the dispersion, however, are lower than those paste viscosities of similarly substituted waxy and normal starches. Etherification of high-amylose starch brings about a marked improvement in its dispersion stability as the tendency to gel or to retrograde is reduced. Greater paste clarity is achieved for this starch by increasing quantities of ethylene oxide, but it is less than that of like derivatives of waxy and normal starches. Also influencing viscos-

ity, clarity, and color of pasted modified high-amylose starch are the pH of the product and the percentage of alkali catalyst used in preparation. The most pronounced changes in the above properties are observed by varying the pH when the catalyst concentrations are relatively high. The presence of salts formed by the neutralization contributes partially to the lowering of the paste viscosity and clarity of hydroxyethylated products of pH 7 in comparison with unneutralized samples. Storage of hydroxyethylated high-amylose starch apparently has only a minimal effect on its paste viscosity and clarity. Comparison of films formed from pastes of the hydroxyethylated derivatives indicates better mechanical properties for the starch containing the highest amount of the amylose fraction.

Quantitative yields of hydroxyethylated high-amylose starch obtained by a practical procedure, together with improved pasting properties over those of the unreacted starch, indicate promise for this derivative of the new starch in industrial applications.

Acknowledgments

The authors are indebted to Mrs. Clara E. McGrew and Mrs. Bonita R. Hopson for ethylene oxide determinations.

Literature Cited

1. Anonymous. High-amylose corn close to commercial reality. J. Agr. Food Chem. **6:** 641–642 (1958).
2. Anonymous. High amylose starch arrives. Chem. Eng. News **36** (50): 54 (1958).
3. Deatherage, W. L., MacMasters, Majel M., Vineyard, M. L., and Bear, R. P. A note on starch of high amylose content from corn with high starch content. Cereal Chem. **31:** 50–52 (1954).
4. Evans, J. W. Modified corn starches. Cereal Science Today **3:** 81–84 (1958).
5. Kerr, R. W. Chemistry and industry of starch (2nd ed.), p. 191. Academic Press, New York (1950).
6. Kesler, C. C., and Bechtel, W. G. Recording viscometer for starches. Ind. Eng. Chem., Anal. Ed. **19:** 16–21 (1947).
7. Kesler, C. C., and Hjermstad, E. T. Starch ethers in original granule form. U. S. Patent No. 2,516,632 (July 25, 1950).
8. Kesler, C. C., and Hjermstad, E. T. Preparation of starch ethers in original granule form. U. S. Patent No. 2,516,633 (July 25, 1950).
9. Kesler, C. C., and Hjermstad, E. T. Cold-water swelling starch ethers in original granule form. U. S. Patent No. 2,516,634 (July 25, 1950).
10. Lortz, H. J. Determination of hydroxyalkyl groups in low-substituted starch ethers. Anal. Chem. **28:** 892–895 (1956).
11. Mazurs, E. G., Schoch, T. J., and Kite, F. E. Graphical analysis of the Brabender viscosity curves of various starches. Cereal Chem. **34:** 141–152 (1957).
12. Rankin, J. C., and Mehltretter, C. L. Process for preparing hydroxyalkylated cereal flour. U. S. Patent No. 2,900,268 (August 18, 1959).
13. Rankin, J. C., Mehltretter, C. L., and Senti, F. R. Hydroxyethylated cereal flours. Cereal Chem. **36:** 215–227 (1959).
14. Schoch, T. J., and Maywald, E. C. Microscopic examination of modified starches. Anal. Chem. **28:** 382–387 (1956).
15. Senti, F. R. Research to utilize amylose. Chemurgic Dig. **17:** 7–12 (1958).
16. Sloan, J. W., MacMasters, Majel M., and Senti, F. R. A note on methods of dispersing fifty-percent-amylose corn starch. Cereal Chem. **36:** 196–198 (1959).

17. Wolff, I. A., Davis, H. A., Cluskey, J. E., Gundrum, L. J., and Rist, C. E. Preparation of films from amylose. Ind. Eng. Chem. 43: 915–919 (1951).
18. Wolff, I. A., Hofreiter, B. T., Watson, P. R., Deatherage, W. L., and MacMasters, Majel M. The structure of a new starch of high amylose content. J. Am. Chem. Soc. 77: 1654–1659 (1955).

A NOTE ON THE DETERMINATION OF GELATINIZATION TEMPERATURES OF RICE VARIETIES[1]

J. V. Halick, H. M. Beachell, J. W. Stansel, and H. H. Kramer[2]

Differences in gelatinization and pasting characteristics of rice varieties as determined on the Brabender Amylograph were recently reported (1). At the concentration (50 g. rice flour and 450 ml. of water) of rice slurry used to obtain the normal amylograph curve, the initial viscosity increase of the rice pastes was ill-defined and covered a range of several degrees. It was recognized that swelling in starch granules begins well before the effect of increased viscosity becomes apparent. Experiments showed that if the rice-to-water ratio were increased (100 g. rice flour and 400 ml. water), the viscosity rise was quite abrupt and the point of departure of the curve from the base line could be taken as a measure of gelatinization temperature. Values for different rices were well defined and highly reproducible and helped to explain differences in cooking and processing behavior.

This note compares gelatinization temperatures obtained by the amylograph method and those found with the more time-honored procedures such as loss of birefringence and estimation of granule swelling. For the microscopic studies ten whole kernels of milled rice were gently macerated with 2 to 3 drops of distilled water with a mortar and pestle. The birefringence end-point temperature (BEPT) determinations were made on the prepared samples according to the method of Pfahler *et al.* (2). This procedure was also used to estimate gelatinization temperature by swelling, except that the starch granules were viewed under ordinary rather than polarized light. The gelatin-

[1] Manuscript received January 15, 1960. Contribution from the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Purdue University, the Texas Agricultural Experiment Station, and the Texas Rice Improvement Association.

[2] Chemist and Research Agronomist, respectively, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beaumont, Texas; and Research Assistant and Professor, respectively, Agronomy Department, Purdue University, Lafayette, Indiana.

ization temperature was taken as that temperature attained when the individual granules had swollen to the bursting point. For the amylograph measurements, a slurry of 100 g. rice flour (fully milled and ground in a Wiley Mill No. 3 to pass a 0.5-mm. screen) and 400 ml. of distilled water was used. The amylograph determinations were made in the spring of 1958 at Beaumont, Texas, and the microscopic determinations at Purdue University the following winter. Amylose contents were determined according to the procedure of Williams *et al.* (3), modified by Deobald.[3]

Gelatinization temperatures and amylose contents of 20 rice varieties grown under uniform conditions at Beaumont, Texas, in 1957, and one commercial sample of glutinous rice (Mochi Gomi) are shown in Table I. In general, the results of the three individual methods

TABLE I

GELATINIZATION CHARACTERISTICS AND AMYLOSE CONTENTS OF RICE VARIETIES

VARIETY	GRAIN TYPE	AMYLOSE	GELATINIZATION TEMPERATURES		
			Amylograph	Granule Swelling	BEPT
		%	°C	°C	°C
Mochi Gomi	Short	0.0	60.0	...	63.0
Caloro	Short	13.9	66.0	66.0	65.0
Asahi	Short	18.1	66.0	68.0	67.0
Arkrose	Medium	16.7	66.0	67.0	68.0
Improved Blue Rose	Medium	18.3	66.0	65.0	62.0
Toro	Long	13.3	66.0	68.0	64.0
Cody	Short	13.6	67.5	69.0	68.0
Magnolia	Medium	11.4	67.5	67.0	65.0
Zenith	Medium	16.4	67.5	68.0	68.0
Calrose	Medium	14.5	67.5	67.0	65.0
Rexark	Long	15.9	67.5	67.0	66.0
Nato	Medium	14.9	69.0	68.0	68.0
TP 49	Long	27.9	69.0	69.0	69.0
Rexoro	Long	28.8	69.0	70.0	69.0
Texas Patna	Long	26.2	70.5	72.0	70.0
Bluebonnet 50	Long	...	72.0	75.0	72.0
Improved Bluebonnet	Long	25.7	72.0	73.0	72.0
Fortuna	Long	22.3	72.0	74.0	73.0
Sunbonnet	Long	24.9	73.5	74.0	72.0
Early Prolific	Medium	12.3	76.5	75.0	75.0
Century Patna 231	Long	14.7	76.5	77.0	76.0

agree well. The coefficient of correlation between the values for the amylograph determination and the BEPT method was +0.939**, and that between the amylograph values and the granule-swelling procedure was +0.955**. These values are considerably greater than the value r = 0.549 required for significance at the 1% level where n = 19.

[3] Private communication (1959) from H. J. Deobald, Food Crops Laboratory, Southern Utilization Research and Development Division, P.O. Box 7307, New Orleans 19, La.

Where sample size is not limiting, greater accuracy can probably be had by the amylograph determination. However, in a breeding program where the sample size is necessarily limited, the value of the BEPT determination is readily recognized. For example, it is possible to obtain sufficient starch for the determinations from the endosperm of a rice kernel and yet save the embryo for later planting if desired.

It is apparent from the figures of Table I, which are arranged in order of inceasing magnitude of gelatinization temperature by the amylograph method, that amylose content of rice has little if any relationship to gelatinization temperature. The correlation coefficient between these two sets of values was calculated to be 0.114, whereas $r = 0.432$ is required for significance at the 5% level.

Acknowledgments

This work was supported in part by grants-in-aid from Campbell Soup Company and General Foods Corporation to the Texas Agricultural Experiment Station, which we gratefully acknowledge.

Literature Cited

1. HALICK, J. V., and KELLY, V. J. Gelatinization and pasting characteristics of rice varieties as related to cooking behavior. Cereal Chem. **36:** 91–98 (1959).
2. PFAHLER, P. L., KRAMER, H. H., and WHISTLER, R. L. Effect of genes on birefringence end-point temperature of starch grains in maize. Science **125:** 441–442 (1957).
3. WILLIAMS, VIRGINIA R., WU, W. T., TSAI, H. Y., and BATES, H. G. Varietal differences in the amylose content of rice starch. J. Agr. Food Chem. **6:** 47–48 (1958).

SEDIMENTATION VALUE AS AN INDEX OF DOUGH-MIXING CHARACTERISTICS IN EARLY-GENERATION WHEAT SELECTIONS[1]

LAWRENCE ZELENY[2], W. T. GREENAWAY[3], GEORGIA M. GURNEY[2], C. C. FIFIELD[3], AND KENNETH LEBSOCK[3]

ABSTRACT

Mixogram curves were made and sedimentation values determined on flours from 159 samples of hard red spring wheat representing F_3-generation lines from a cross between a high-protein, strong-gluten variety, Conley, and a low-protein, weak-gluten strain, P.I. 56219-12, used for breeding purposes because of its sawfly resistance. In addition, two samples each of the parent wheats were used in the study. Sedimentation values of the strong and weak parent wheats were 69 and 26, respectively, and all but one of the F_3-generation lines had values intermediate between these two extremes. Highly significant correlations were found between sedimentation values, mixing times, mixing tolerances, and weakening angles as determined from the mixograph curves. Correlations between protein content and these dough-mixing properties were low and not significant.

The sedimentation test may provide a useful and simple tool for making early-generation wheat selections on the basis of dough characteristics. By using a semimicro technique, it should be possible to apply the test to the wheat from individual plants.

Gluten quality is a particularly important factor to consider in breeding hard winter and spring wheats since varieties with weak gluten characteristics are unacceptable for the production of bread flour. Wheats having long mixing time usually are preferred, largely because mixing time is rather closely associated with mixing tolerance. Long mixing tolerance is in reality the most sought-after quality characteristic in hard wheat breeding programs in the United States.

Variation among wheats in gluten quality is for the most part genetically controlled, and the effectiveness of selection for desirable types depends upon evaluation procedures available. The lack of any satisfactory simple method for judging dough-making characteristics in early generations makes it necessary to carry many lines for additional generations. The efficiency with which high-quality wheats can be isolated from a hybrid lot of material might be increased markedly through the development and use of suitable methods for judging quality in early generations, such as the F_3 generation.

The micromilling technique of Seeborg and Barmore (6) makes it

[1] Manuscript received December 23, 1959. Contribution from the Agricultural Marketing Service and the Agricultural Research Service of the U. S. Department of Agriculture, in cooperation with the North Dakota Agricultural Experiment Station.

[2] Grain Division, Agricultural Marketing Service, U. S. Dept. of Agriculture.
[3] Crops Research Division, Agricultural Research Service, U. S. Dept. of Agriculture.

possible to judge certain important milling characteristics on 5 g. of wheat. Protein content can be determined on 1 g. of wheat, or even on individual kernels if necessary. Gluten quality evaluation, however, is usually accomplished by means of baking tests or from a study of dough characteristics as determined with a recording dough mixer. Both of these procedures usually require a relatively large amount of wheat and therefore cannot normally be applied to the early generations in the breeding program. It should be noted, however, that micromilling techniques have been developed by Geddes and Frisell (3) and by Finney and Yamazaki (2), and a micro-baking technique by Shogren and Shellenberger (8).

The purpose of this paper is to investigate the possible usefulness of the sedimentation test in wheat breeding for the purpose of selecting promising lines from early generations. The studies reported are confined to two parent wheats of widely different dough-making characteristics and 159 F_3 lines resulting from a cross between these varieties.

Materials and Methods

Wheat samples used for this study consisted of 159 F_3 lines taken from an unselected population from a cross between the variety Conley and the strain P.I. 56219-12 introduced from Portugal. Conley is a hard red spring wheat of long mixing time, long mixing tolerance, and excellent bread-baking strength. P.I. 56219-12 is a hard red spring wheat introduction useful for breeding purposes primarily because of its solid stem and consequent sawfly resistance (a characteristic not shared by Conley), but it is of relatively short mixing time and tolerance. Included also in the study were two samples of each parent variety, which in each case were grown in separate rod rows. All of the wheats were grown in the same plot at the North Central Experiment Station, Minot, North Dakota, in 1956. About 160 g. of each sample were available for milling.

The samples were tempered to a moisture content of 16% and milled to straight-grade flours on the Allis-Chalmers experimental flour mill. Because of the very small size of the samples, flour-yield data were considered to be of no particular significance.

Mixograms were made with the National Manufacturing Company's Mixograph. The weight of the flour used was 35 g. calculated to a 14.0% moisture basis. The absorptions used were calculated by use of the regression equation and according to the procedure described by Johnson *et al.* (4). All tests were made at temperatures within the range of 25°–27°C. Mixing time and weakening angle

were determined by the method of Johnson *et al.* (4). Mixing tolerance was determined as the horizontal distance between the centers of the ascending and descending parts of the mixogram through the bottom of the band at its peak point (see Fig. 1). Obviously these measurements are not completely objective, but they were all made in a uniform manner by the same experienced operator and should be reasonably comparable.

Sedimentation value was determined by the method of Zeleny (9) as modified by Pinckney *et al.* (5) and described in *Cereal Laboratory Methods* (1). The flour samples milled with the Allis-Chalmers experimental flour mill were used for the sedimentation tests. Specific sedimentation value was determined by dividing the sedimentation value by the percentage of protein in the flour, calculated to a 14.0% moisture basis. The sedimentation value is influenced both by quantity and "strength" of the gluten protein, while the specific sedimentation is essentially an index of gluten strength without regard to protein content.

Protein content was determined by the Kjeldahl method.

Results

Mixograms for one of the samples of each of the parent wheats are shown in Fig. 1. Some of the F_3 lines gave mixograms similar to those of each of the parents, but most of them gave mixograms intermediate in character.

The pertinent mixogram data as well as the sedimentation and protein data for the two parent wheats are shown in Table I.

The distribution of sedimentation values among the samples and the corresponding mixogram data by class averages are shown in Table II.

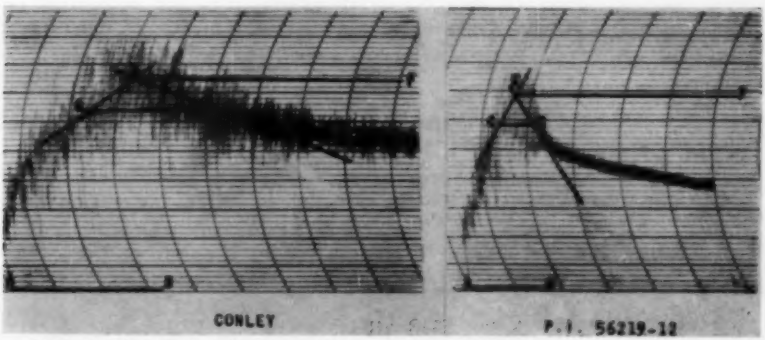


Fig. 1. Mixographs of parental wheats. AB = mixing time; CD = mixing tolerance; DEF = weakening angle.

The more important statistical relationships among mixogram, sedimentation, and protein data are shown in Table III.

TABLE I

MIXOGRAM CHARACTERISTICS, SEDIMENTATION, AND PROTEIN DATA FOR THE FLOURS FROM THE TWO SAMPLES OF EACH OF THE PARENT WHEATS

	Conley			P. I. 56219-12		
	A	B	Av.	A	B	Av.
Mixing time, minutes	3.25	3.00	3.12	1.75	1.50	1.62
Mixing tolerance, minutes	2.75	3.50	3.12	0.75	0.75	0.75
Weakening angle, degrees	20	32	26	58	55	56
Sedimentation value	69	69	69	26	26	26
Specific sedimentation	4.4	4.4	4.4	2.1	2.1	2.1
Protein, %	15.8	15.5	15.6	12.5	12.4	12.4

TABLE II

SEDIMENTATION VALUES AND THE DISTRIBUTION OF MIXOGRAM DATA BY CLASS AVERAGES FOR 163 SAMPLES OF WHEAT REPRESENTING TWO SAMPLES EACH OF THE PARENT WHEATS CONLEY AND P. I. 56219-12 AND 159 SAMPLES REPRESENTING F_3 LINES OF THE CROSS

Sedimentation Value	No. of Cases	Av. Mixing Time	Av. Mixing Tolerance	Av. Weakening Angle
		minutes	*minutes*	*degrees*
25-29	5	1.40	0.60	61
30-34	9	1.53	0.72	58
35-39	31	1.68	0.84	53
40-44	31	1.82	1.03	50
45-49	20	2.01	1.18	44
50-54	19	2.25	1.50	41
55-59	15	2.02	1.28	44
60-64	21	2.33	1.85	35
65-69	12	2.81	2.19	33

TABLE III

STATISTICAL ANALYSIS OF DATA

Factors	Correlation Coefficient	Standard Error of Estimate
Sedimentation value vs. mixing time	0.61**	0.42 minutes
Sedimentation value vs. mixing tolerance	0.74**	0.38 minutes
Sedimentation value vs. weakening angle	−0.62**	9.2°
Specific sedimentation vs. mixing time	0.67**	0.39 minutes
Specific sedimentation vs. mixing tolerance	0.78**	0.35 minutes
Specific sedimentation vs. weakening angle	−0.69**	8.5°
Protein content vs. mixing time	−0.01	0.53 minutes
Protein content vs. mixing tolerance	0.14	0.56 minutes
Protein content vs. weakening angle	0.10	11.8°
Protein content vs. sedimentation value	0.52**	9.4 ml.
Mixing time vs. mixing tolerance	0.80**	0.33 minutes

Discussion

Mixogram, sedimentation, and specific sedimentation values for the 159 F_3-generation wheats all covered wide ranges, and almost all of the values fell at intermediate points between the high values of the Conley parent and the low values of the P. I. 56219-12 parent. Highly significant correlations were found between both sedimentation value and specific sedimentation value, on the one hand, and mixing time, mixing tolerance, and weakening angle on the other hand. Generally speaking, mixing time and mixing tolerance were closely associated, as evidenced by the high correlation between these two factors. The correlations between protein content and mixogram characteristics were low and not significant.

Of the 159 F_3-generation wheats used in this study 17, or 11%, had mixing times of 2.75 minutes or over; and 19, or 12%, had mixing tolerances of 2.00 minutes or over. Thirty-one, or 19%, of the 159 wheats had sedimentation values of 60 or over. This small group of high-sedimentation wheats included 59% of all of the samples having long mixing times (2.75 minutes or over) and 79% of all of the samples having long mixing tolerances (2.00 minutes or over). It is also interesting to note that although 59% of the 159 F_3-generation wheats had sedimentation values of less than 50, only three of the seventeen long-mixing-time wheats and none of the nineteen long-mixing-tolerance wheats fell into that group. Thus in this instance it would be possible to segregate reasonably well the great majority of the wheats with the most desirable mixogram characteristics by using the relatively simple sedimentation test as the basis for selection. More than half of the wheats could be eliminated on the basis of sedimentation values without sacrificing a single one of the wheats with long mixing tolerance.

Although complete segregation of the wheats with the most desirable mixogram characteristics obviously cannot be made by means of the sedimentation test, it is interesting to speculate as to whether the sedimentation test itself may not provide a more reliable guide for making wheat selections than does the mixograph or any other recording dough mixer. The data of Pinckney *et al.* (5) indicate that the sedimentation value is a more reliable index of bread loaf volume than is the farinogram. In a survey of the quality of wheats imported by several European countries over an 18-month period and reported by Shellenberger (7), the sedimentation value was found to be a better measure of bread-baking quality ("quality score") than was either protein content, valorimeter value (obtained by the farinograph), or "W" value (obtained by the alveograph).

A total of 361 shipments of wheat produced in 16 different countries was included in this survey.

The greatest advantage of the sedimentation test in evaluating early-generation lines in wheat-breeding programs may prove to be the very small amount of material required. At least 50 g. of wheat are required to obtain sufficient flour for the mixogram, while about 20 g. of wheat are sufficient for making a single sedimentation test by the established method. Preliminary experiments have been made which indicate that sedimentation tests may be made reliably by means of a semi-micro technique using only 2 g. of wheat. In these trials the standard sedimentation test procedure was used except that the size of the flour sample and of the portions of all reagents was reduced to one-tenth, and 10-ml. rather than 100-ml. graduated cylinders were used. Sedimentation values so obtained and multiplied by 10 closely approximated the values obtained by the standard procedure. This technique should permit making tests on the grain from individual wheat plants leaving a sufficient amount of the wheat for planting. Sedimentation tests may be made in an average time of 5 minutes per sample starting with the original wheat when the prescribed milling procedure (1,5) is used.

Acknowledgments

The authors wish to acknowledge the services of Tyler F. Hartsing who milled the 163 samples of wheat used in this study, and of Reba E. Renn who assisted in making the mixograms.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. Finney, K. F., and Yamazaki, W. T. A micromilling technique using the Hobart grinder. Cereal Chem. **23:** 484–492 (1946).
3. Geddes, W. F., and Frisell, B. An experimental flour mill for 100-gram wheat samples. Cereal Chem. **12:** 691–695 (1935).
4. Johnson, J. A., Swanson, C. O., and Bayfield, E. G. The correlation of mixograms with baking results. Cereal Chem. **20:** 625–644 (1943).
5. Pinckney, A. J., Greenaway, W. T., and Zeleny, L. Further developments in the sedimentation test for wheat quality. Cereal Chem. **34:** 16–25 (1957).
6. Seeborg, E. F., and Barmore, M. A. A new five-gram milling-quality test and its use in wheat breeding. Cereal Chem. **34:** 299–303 (1957).
7. Shellenberger, J. A. Survey of the quality of European wheat imports. Kansas Agr. Exp. Sta. Bull. 396 (1958).
8. Shogren, M. D., and Shellenberger, J. A. The design, construction and use of micro baking equipment. Cereal Chem. **31:** 475–482 (1954).
9. Zeleny, L. A simple sedimentation test for estimating the bread-baking and gluten qualities of wheat flour. Cereal Chem. **24:** 465–475 (1947).

COMMUNICATION TO THE EDITOR

Presence and Probable Role of Thioctic Acid in Wheat Flour

DEAR SIR:

Chemists have long sought an explanation of the phenomena involved in the oxidation of flour, but no acceptable theory has been proposed. It is generally believed (3,5,9) that some of the sulfhydryl groups of the flour proteins are oxidized to form disulfide bridges between the peptide chains of the flour proteins or that an exchange between sulfhydryl and disulfide bonds of the proteins is involved. Evidence (2,7) would also indicate that some compound in the lipid fraction (which is closely associated with the protein) is somehow concerned in the oxidation. But the site of the reactive sulfhydryl groups, the sequence of reactions, and the over-all mechanism have remained obscure.

We have identified thioctic acid in the acid-hydrolyzed extract of flour. We believe this compound is concerned in the maturing of wheat flour.

Evidence of the presence of thioctic acid is as follows: Glacial acetic acid was added to a water extract of a straight-grade flour (37 g.) to give a 5% solution (pH 3). The solution was heated 45 minutes in a boiling-water bath and extracted with 30 ml. n-butanol (centrifugation was necessary to destroy colloidal dispersion). The clear butanol extract gave an absorbance at 334 $m\mu$ of 0.40. Addition of 0.01 ml. of tertiary butyl hydroperoxide reduced this value to 0.30 in 17 minutes. Thioctic acid has an absorption maximum at 334 $m\mu$ which is lost on oxidation to the monoxide or further stages (1).

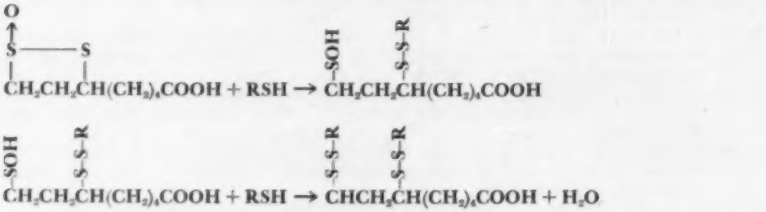
An extract of flour (250 g. flour and 500 ml. water) was made 1.0*N* with hydrochloric acid and heated 3 hours in a boiling-water bath. The hydrolysate was extracted with 150 ml. benzene and the benzene solution was then extracted with two 100-ml. portions of 5% sodium bicarbonate. The bicarbonate solution was acidified with 40 ml. concentrated hydrochloric acid and then extracted with 50 ml. benzene. One milliliter of the benzene extract was spotted on Whatman No. 1 paper and chromatographed (ascending), employing 1% acetic acid as solvent. After immersion in 0.1*N* potassium permanganate, a spot showed an R_f value of 0.7. A drop of 30% hydrogen peroxide added to 2 ml. of the benzene extract and similarly chromatographed gave a spot at $R_f = 0.84$. Synthetic dl-thioctic acid gives

identical values under these conditions. The same procedure, employing water-saturated butanol, gave R_f values of 0.95 for the original and 0.63 for the oxidized solution. Literature reports (6) values of 0.9 for unoxidized thioctic acid and 0.6 for oxidized thioctic acid (thioctic monoxide).

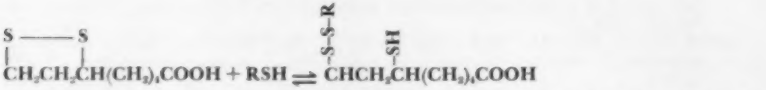
We have also found that, although parachloromercuribenzoate (PCMB) reacts with free −SH groups, these −SH groups can be measured in the presence of PCMB using the amperometric procedure of Kolthoff, Stricks, and Morren (4). Equal titration values for −SH are given in the presence or absence of PCMB with mercuric chloride as titrant. Presumably, mercuric chloride displaces the PCMB from the sulfhydryl groups, the mercuric chloride having a stronger affinity for −SH than PCMB. This provides a means of protecting −SH groups from the time of their solution to the time of measurement. Use is made of this technique in the measurement of the sulfhydryl groups of flour.

Ten grams of a straight-grade, spring wheat flour were dispersed in 200 ml. of 8*M* urea (purged of oxygen) and then made 0.05*M* to borax and 0.5*M* to potassium chloride. The dispersion was amperometrically titrated with 0.001*M* mercuric chloride using the rotating platinum electrode. A value of 0.0018% −SH was obtained. The same procedure, using 200 ml. 8*M* urea containing 5 micromoles PCMB, gave a value of 0.0020% −SH. The water-soluble −SH measured 0.0009% (quenched with PCMB) and 0.0003% (unquenched). When PCMB is used as a quenching agent, the sulfhydryl content of flour is found to be about equally divided between the water-soluble proteins (albumins and globulins) and the more insoluble gluten proteins. The higher value of −SH in the presence of PCMB is consistently observed in the measurement of total −SH of flour. Since the possibility of air oxidation was excluded by careful purging of all solutions with nitrogen, there is some other factor which lowers the −SH content of flour in the absence of a blocking agent. The divergence of values reported in the literature for water-soluble −SH groups in flour may be due to oxidation of the −SH groups by thioctic monoxide. Other investigators (8) have demonstrated a higher value of flour −SH in the cold rather than at room temperature. This has been confirmed in this laboratory. The monoxide of thioctic acid could, conceivably, be the compound which reacts with flour −SH at room temperature, lowering its measurement but decreasing it less at lower temperatures because of less reactivity and not lowering the measurement at all in the presence of a blocking agent such as PCMB.

One could expect the following two-stage reactions:



The following reaction reported by Calvin (1) to be rapid and reversible at pH 8 may also occur at the pH of dough.



Presumably all the sulfhydryl groups compete for the thioctic monoxide sites.

The extent of crosslinking and its consequent effect on the physical properties of a given dough would depend on:

a) The number of thioctic monoxide sites available,
b) The total number of sulfhydryl groups and their distribution, and
c) The working of the dough and the exchange of sulfhydryl with disulfide groups.

The addition of an oxidant, either air or one of the commonly employed maturing agents, would increase the number of thioctic monoxide sites and these, in turn, would decrease the number of reactive SH groups. The correct amount of oxidant and work would produce the proper balance to give the crosslinking in the dough most desirable for optimum baking characteristics.

The sodium salt of thioctic acid and its monoxide used at 20 mg. percent markedly increase the extensibility of dough and decrease the resistance to extension, as measured on the extensigraph.

We are attempting to isolate thioctic acid from wheat flour. The fact of its presence and its ease of oxidation allows a more reasonable explanation of flour maturing than has been possible heretofore, but the over-all mechanism needs further proof.

LELAND DAHLE
BETTY SULLIVAN

Research Laboratories,
Russell-Miller Milling Company,
Minneapolis, Minnesota

August 9, 1960

Literature Cited

1. CALVIN, M. Chemical and photochemical reactions of thioctic acid and related disulfides. Federation Proc. **13:** 697–711 (1954).
2. COOKSON, M. A., and COPPOCK, J. B. M. The role of lipids in baking. III. Some breadmaking and other properties of defatted flours and of flour lipids. J. Sci. Food Agr. **7:** 72–87 (1956).
3. GOLDSTEIN, S. Sulfhydryl- und Disulfidgruppen der Klebereiweisse und ihre Bezeihung zur Backfähigkeit der Brotmehle. Mitt. Lebensm. Hyg. Bern **48:** 87–93 (1957).
4. KOLTHOFF, I. M., STRICKS, W., and MORREN, L. Amperometric mercurimetric titration of sulfhydryl groups in biologically important substances. Anal. Chem. **26:** 366–372 (1954).
5. PENCE, J. W., MECHAM, D. K., and OLCOTT, H. S. Nutrients in wheat products; review of proteins of wheat flour. J. Agr. Food Chem. **4:** 712–716 (1956).
6. REED, L. J., and DE BUSK, BETTY G. Enzymatic synthesis of a lipoic coenzyme. Federation Proc. **13:** 723–731 (1954).
7. SMITH, D. E., VAN BUREN, J. P., and ANDREWS, J. S. Some effects of oxygen and fat upon the physical and chemical properties of flour doughs. Cereal Chem. **34:** 337–349 (1957).
8. SOKOL, H. A., MECHAM, D. K., and PENCE, J. W. Further studies on the determination of sulfhydryl groups in wheat flours. Cereal Chem. **36:** 127–133 (1959).
9. SULLIVAN, BETTY. Proteins in flour. J. Agr. Food Chem. **2:** 1231–1234 (1954).

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, of fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for Cereal Chemistry.

Manuscripts for publication, and correspondence directly pertaining to them, should be sent to the Editor-in-Chief, CEREAL CHEMISTRY, 1955 University Ave., St. Paul 4, Minn. All other correspondence, including circulation, advertising, or other business, should be directed to the Managing Editor at the above address.

Manuscripts of published papers will be kept on file for one year. After that time they will be destroyed unless other instructions have been received from the author. Original graphs, etc., and negatives of all illustrations are returned to the author immediately upon publication.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangement and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" *(Trans. Am. Assoc. Cereal Chemists* 6:1–22. 1948) amplifies the following notes.

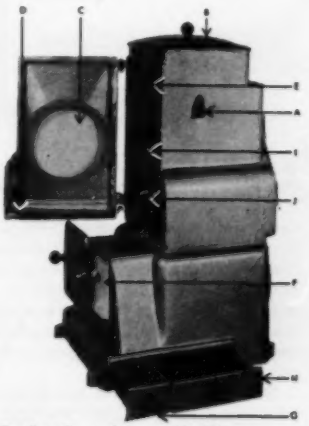
Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

NOW! TABLE TOP MICRO-ROLLER TEST MILLING

C. W. BRABENDER QUADRUPLEX PILOT MILL

On the same wheat, there is no greater Farinogram difference on Quadruplex milled and commercially milled flour than between the flour from two commercial mills. The new C. W. Brabender Quadruplex Mill yields an average of 69% Hungarian. Grinding rate is 35 grams/minute — hopper holds 250 grams. Because the roll setting is fixed for all types of wheat, a skilled operator is not required. For purposes of research on wheat grindability and yield, exchange rolls with various gaps and corrugations are available and interchangeable in one minute.

The quadruplex system of corrugated precision ground rolls—*corrugated after roll hardening.*



A. Feeder Control Knob
B. Feed Hopper
C. Filter Silk Screen
D. Filter Dust Collector Tube
E. Removable Cover Plate For Mill Roll Cleaning
F. Sifter Chamber
G. Bran Drawer
H. Flour Drawer
I. Aspirator Blower
J. Aspirator Adjustor

Self-contained. 16"x19"x 28". Weight 41 lbs. Operates on 220/440 Volt., 3 phase, 60 cycle.

C.W. Brabender

INSTRUMENTS, INC., SOUTH HACKENSACK, N. J.
50 EAST WESLEY STREET, DIAMOND 3-8425

Write C. W. Brabender, president. His application knowledge is yours without obligation.

How *Sterwin* Puts More Profit Into Food Products!

If your food product requires enrichment, color, flavor . . . then Sterwin can help you save time, money, labor . . . so give you more profit.

For Sterwin offers unique extras in vitamins, colors, vanillin and these superior ingredients are fitted into your production picture by experts with years of practical and technical experience. These Sterwin Technically Trained Representatives have cut time, expense, production steps for manufacturers producing a widely varied range of food products: flour, candy, bread, margarine, soda, fruit juice, cereals . . . to name just a few.

Sterwin ingredients are delivered when you want them, in the quantity you need. Keeping delivery promises is a Sterwin tradition that helps keep your production at top efficiency.

For new production set-ups or to improve an existing one . . . see your local Sterwin Representative . . . costs you nothing . . . can profit you plenty.

You get these extras when you buy:

STERWIN VITAMINS . . . *the most complete line in the field . . . immediate delivery in any quantity.*

STERWIN COLORS . . . Parakeet Certified F.D.&C. . . . *backed by a highly experienced technical staff to help you with your color problems.*

STERWIN VANILLIN . . . pure, ZIMCO® U.S.P. Vanillin . . . *unlimited supply of basic material from world's largest vanillin producer.*

Sterwin Chemicals inc.

Subsidiary of Sterling Drug Inc.

1450 BROADWAY, NEW YORK 18, NEW YORK

STOCKS AVAILABLE AT: Atlanta, Buffalo, Dallas, Denver, Evanston, Ill., Kansas City, Mo., Los Angeles, Menlo Park, Calif., Minneapolis, Portland, Ore., Rensselaer, N. Y., St. Louis

NOW AVAILABLE

THE FARINOGRAPH HANDBOOK

- The Farinograph
- Theoretical Aspects of the Farinograph
- Types of Farinograph Curves
- Factors Influencing Farinograms
- Techniques and Uses of the Farinograph

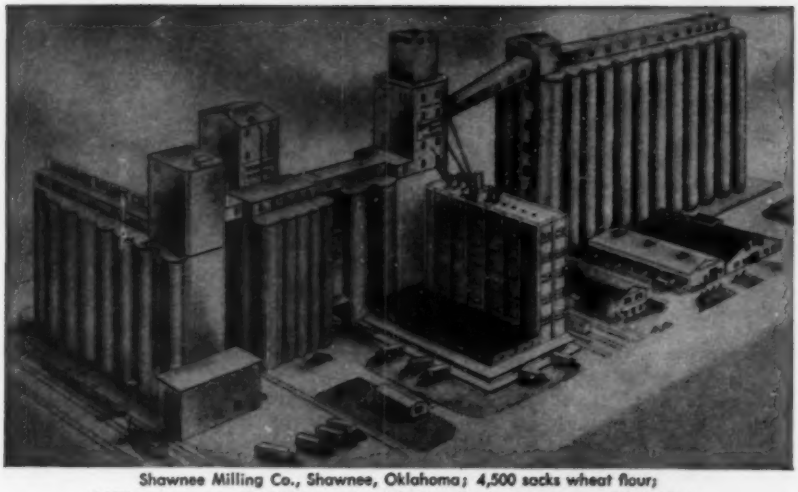
Based on lectures presented at the AACC's 1st Farinograph Workshop, May 5-6, 1960, Chicago, Illinois. The only complete manual on the Farinograph ever published. A composite of the knowledge and experience of leading experts in the field of physical dough testing. Sponsored by the Physical Testing Methods Committee of the American Association of Cereal Chemists.

Price $5.00

ORDER FROM

AMERICAN ASSOCIATION OF CEREAL CHEMISTS

1955 University Avenue **St. Paul 4, Minnesota**



Shawnee Milling Co., Shawnee, Oklahoma; 4,500 sacks wheat flour; 1,200 sacks corn meal; 250 tons mixed feed; mill elevator storage 2,500,000 bu.

SHAWNEE MILLING COMPANY SCALPS MILLING COSTS

With W&T Flour Treatment

At Shawnee Milling, mill managers control some of their costs with dependable Wallace & Tiernan equipment and processes. They know that quality, cost, and good running time go hand in hand—that they can depend upon W&T to keep its bleaching and maturing processes running —that stoppages won't turn flour into the "dirty" bin for costly reblending.

And Shawnee uses W&T's flour treatment not only for dependability but for performance:

- Dyox® for fresh, sharp chlorine dioxide gas, made as it is used... precisely metered as a gas not a liquid.
- Novadelox® for best color removal, ease of application, consistent results.
- Beta Chlora® machines for pinpoint pH control with one, easily-read setting.

Shawnee Milling Company is only one of the many milling companies using W&T flour treatment. If your mill is not one of these, investigate the advantages of Wallace & Tiernan's complete flour service.

NOVADEL FLOUR SERVICE DIVISION
WALLACE & TIERNAN INCORPORATED
25 MAIN STREET, BELLEVILLE 9, NEW JERSEY
REPRESENTATIVES IN PRINCIPAL CITIES

N-118.20